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DOUBLE VAGOTOMY IN RELATION TO RESPIRATION.

By G. V. ANREP and ADLI SAMAAN.

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Pavlov [1895, 1896] and his pupils, Cachcovsky [1899] and Cheshcov [1902], succeeded in keeping dogs alive after simultaneous double vagotomy in the neck. This was the first case reported in the literature of a prolonged survival of dogs operated on in this way. One of these animals survived in Pavlov's laboratory for 19 months, and died from an accidental cause. Pavlov ascribes the success of his experiments to the fact that œsophagostomy and gastrostomy were established in all his dogs before their vagi were cut. The œsophagostomy safeguarded the animals against the penetration of regurgitated food into the respiratory passages. The diet of the animals had to be carefully selected, and the stomach had to be washed out at regular intervals through the gastric fistula, in order to prevent auto-intoxication due to the stagnation of semi-digested food in the stomach. Pavlov and his co-workers give a very detailed description of the changes in the respiratory activity of these vagotomized dogs. In all the animals, immediately after the section of both cervical vagi, the rate of respiration fell to 4–8 per minute. However, this slowing down of breathing gradually disappeared and, towards the second or third week after the operation, the respiratory rate returned almost to normal. After this the rate gradually diminished again and finally settled at 4–8 per minute, the respiration remaining at this slow rate up to the death of the animals. The immediate slowing down of the breathing, its temporary acceleration and then the final slowing down were typical of all operated animals. The respiratory slowing down is ascribed by Pavlov to the section of the sensory pulmonary fibres of the pneumogastric nerves. The temporary acceleration on the second to the fourth week after the operation is tentatively explained as being due to excitations arising in the central ends of the cut vagi during the process of healing at the place of section. Thus Pavlov's observations on the slow breathing of surviving vagotomized animals correspond with the results of all the previous authors in vivisection experiments. In

explaining this phenomenon Pavlov is also in agreement with the older observers.

This classical explanation of the slow breathing of vagotomized animals has lately been subjected to considerable criticism. Boothby and Shamoff [1915] claim to have performed a complete intra-thoracic denervation of the lungs by cutting the nerve filaments at the root of each lung while the trunks of the vagi were left intact. The animals survived for several months and showed no change either in the rate or in the depth of respiration. Moreover Boothby and Berry [1915] noticed that, in the same dogs, distension of the lungs by positive pressure produced the usual cessation of respiration (Hering-Breuer's reflex [1868]), an effect which invariably disappears after section of the cervical vagi. Thus these animals present a striking contrast to those which had their vagi cut in the neck.

Sharpey-Schafer [1919, 1932], working chiefly on cats, expressed the opinion that the slowing down and deepening of the respiratory movements in cervical vagotomy is not due to the section of the sensory pulmonary fibres, but to an increased mechanical resistance caused by the bilateral paralysis of the laryngeal muscles and the falling together of the thyro-arytenoid ligaments, the laryngeal paralysis obviously resulting from the section of the inferior (recurrent) laryngeal fibres. As a result of a few experiments performed on dogs and rabbits, Sharpey-Schafer ascribes the respiratory slowing down in these animals to the same cause.

J. F. and C. Heymans [1928], working on dogs, also deny that the respiratory slowing down is due to the denervation of the lungs. They used the same technique as Head [1889 *a*, *b*], and fundamentally their experiments are very similar to those of Moore [1927]. They cut one vagus in the neck and kept the animal alive by rhythmic insuflation of the denervated lung. The other, innervated, lung was collapsed. Under these conditions it was noticed that, on cutting the cervical vagus, *i.e.* the vagus on the side of the collapsed lung, the respiration assumed the typical vagal rate. In interpreting these experiments the authors assume that, in the case of denervation of one lung and collapse of the other innervated lung, all pulmonary influences upon the respiratory rate are abolished, and therefore the slow vagal type of breathing cannot be explained by a destruction of the pulmonary innervation. They regard the respiratory slowing down as due to the removal of cardio-aortic impulses, and conclude that what they call the "respiratory vagal tone" is of cardio-aortic and not of pulmonary origin.

The following are the various theories which have been advanced up to the present in order to explain the cause of the respiratory slowing down after double cervical vagotomy:

(1) The removal of the pulmonary control of the respiratory rate (the generally accepted view).

(2) Any other cause but the denervation of the lungs (Boothby and Shamoff).

(3) An increased resistance in the respiratory passages due to the paralysis of the laryngeal muscles (Sharpey-Schafer).

(4) The removal of a cardio-aortic control of the respiratory rate (J. F. and C. Heymans).

The experiments described in the present communication were performed in order to test these different theories. The observations deal with the respiratory changes which develop immediately after vagotomy, as well as with changes observed within a few days (up to the ninth day) after the operation. Adult dogs and cats were used for the experiments. The following anæsthetics were employed: chloroform and ether, chloralose (0·05–0·075 g. per kg. intravenously), sodium luminal (0·05 g. per kg. intravenously) and urethane (0·30 g. per kg. subcutaneously). A small dose of morphine hydrochloride (0·003–0·005 g. per kg. subcutaneously) was injected in some experiments before anæsthesia. All animals which were allowed to recover from the anæsthesia were operated upon aseptically.

The laryngeal theory of the respiratory slowing down.

As a result of a great number of experiments performed chiefly on cats, Sharpey-Schafer, using various indirect methods, has come to the conclusion that the slow respiratory rhythm which develops after double cervical vagotomy is due to the paralysis of the laryngeal muscles, and not to the denervation of the lungs as was hitherto supposed. According to this observer, section of the vagi in a tracheotomized cat or dog, or after cauterization of the thyro-arytenoid ligaments, fails to produce any permanent change in the respiratory rate. Without this precaution, an obstruction to inspiration is caused by the falling together of the thyro-arytenoid ligaments, and this increased resistance is the cause of the slow vagal type of respiration. This conclusion could easily have been tested by a section of the inferior (recurrent) laryngeal nerves while the other vagal nerves are left intact. This direct test, however, was not performed. The following experiments were carried out in order to test Sharpey-Schafer's conclusion.

Both inferior laryngeal nerves were exposed in the neck, and the respiratory movements were recorded either by a stethograph placed round the lower part of the thorax or by Marey's tambour fixed to the larynx or to the chest wall. In some experiments the larynx was kept intact and in others it was dissected, separated from its pharyngeal connections, and exposed so that the movements of the thyro-arytenoid ligaments could be observed. Section of one or both inferior laryngeal nerves had no effect whatever on the depth or rate of the respiratory movements. The complete paralysis of the laryngeal muscles was verified in every experiment by direct observation. These experiments were repeated on many occasions on dogs and cats, and no difference was noticed in the result. In several dogs a length of 1 or 2 cm. of each inferior laryngeal nerve was excised in the neck under aseptic conditions; these animals were kept alive for several months. Except for the usual change in the voice, no other disturbances were apparent, and in no case was the rate of respiration changed.

In another series of experiments a cannula was tied directly into the trachea, or a wide rubber tube was passed from the mouth downwards through the rima glottidis, in order to prevent the vocal cords from possibly causing an obstruction to the movements of air in the respiratory passages. The respiratory movements were recorded as before. The inferior laryngeal and the vago-sympathetic nerves were exposed on both sides of the neck. It was noticed that, while the section of the recurrent laryngeal nerves had not the slightest effect on the rate of respiration, the section of the vago-sympathetic nerves immediately produced the characteristic slowing down. The slow rate of breathing remained unchanged whether the rubber tube inserted through the glottis was left in position or removed (Fig. 1 *A* and *B*).

If the straight limb of a T-piece tube is introduced into the trachea, the animal can be made to breathe either freely through the open limb of the T-piece or through the larynx. A vagotomized animal does not change its rate of respiration in either case (Fig. 1 *C*).

Sharpey-Schafer believes that the slow rate of breathing in vagotomized animals is due to an inspiratory collapse of the thyro-arytenoid ligaments. It is, however, easy to show that, while an increased resistance to expiration brings about a slowing down of breathing [Davis, Haldane and Priestley, 1919], an increased resistance to inspiration leaves the respiratory rate almost unchanged. For instance in one experiment the trachea of the dog was attached to two Boulitte respiratory valves, each of which was in turn connected with a Müller's water valve. By

lowering or raising the tube in the inlet or outlet Müller's valves, the inspiratory or the expiratory resistance respectively could be changed. The animal was breathing normally at the rate of 23–26 per minute. On increasing the inspiratory resistance to 11 cm. of water, the respiratory rate remained almost unchanged, while even a small increase of the

Fig. 1. Dog 7 kg. Chloralose. The respiratory movements of the chest are recorded by a stethograph connected to a Marey's tambour. Inspiration upwards. *A*. The section of the right and then the left inferior laryngeal nerve is indicated by arrows. The respiratory rate (24 per minute) was unaffected by the section. It remained constant for 90 min. when the right vagus was cut in the neck. A few minutes after the section the respiration assumed the rate of 20 per minute and remained constant for another 90 min. at which point (at the arrow in *B*) the left vagus was cut in the neck. The respiration immediately slowed down to 5–6 per minute and remained unchanged until the end of the experiment. A T-shaped cannula was introduced into the trachea which allowed the dog to breathe either through the open limb of the cannula or through the larynx. *C*. Taken 60 min. after the previous tracing. During the whole time the animal was breathing through the open limb of the cannula. At the arrow it was made to breathe through its larynx. The respiratory rate remained unchanged. This and the following tracings should be read from left to right. Time in all tracings in 5 sec.

expiratory resistance to 4 cm. of water reduced the rate from 26 to 16 per minute. This effect of increased resistance to expiration is, however, noticed only when the vagi are left intact. After section of the cervical vagi, an increase in the resistance fails to produce any effect on the rate of the respiration, unless the resistance is so great that it produces

asphyxiation of the animal. Since the increase in the expiratory resistance is effective only in the presence of the vagi, it cannot be regarded as a cause of the slow respiration in absence of the vagi.

The experiments described above fail to support Sharpey-Schafer's explanation. In adult dogs the paralysis of the laryngeal muscles cannot be considered as the cause of the respiratory slowing down following double cervical vagotomy.

The cardio-aortic theory of the respiratory slowing down.

J. F. and C. Heymans consider that the "respiratory tone" of the vagus nerve is of cardio-aortic origin, and that the slow vagal type of respiration is due to section of the cardio-aortic nerve fibres. No direct evidence in support of this view is, however, provided. They base their theory on the observation that section of the cervical vagi during a deflated condition of the lungs leads to a slowing down of the respiration, similar to that when the vagi are cut during normal movements of the lungs and of the respiratory muscles. But since deflation of the lungs normally produces a considerable acceleration of the respiratory movements, it would be expected that section of the vagi would lead to a comparatively greater slowing down when made during deflation of the lungs than when made on an animal which breathes spontaneously, or whose lungs are rhythmically insuflated. An inflation of the lungs produces the well-known Hering-Breuer's arrest or considerable slowing down of the respiratory movements. The extent of the effect of double vagotomy is at least in part dependent on the degree to which the inflations of the lungs have been inhibiting the respiration before the section.

The theory of the alleged cardio-aortic origin of the "respiratory vagal tone" could be directly tested by observing the effect on respiration of a complete destruction of the cardio-aortic innervation. Moreover, if this theory is correct, section of the cervical vagi after a cardio-aortic denervation should produce no effect on respiration. The chest of the animal was opened along the mid-sternal line and the internal mammary blood vessels were cut between ligatures. The depth and rate of the artificial ventilation was adjusted to prevent the development of apnœa, so that the animal performed respiratory movements during the whole experiment. These movements, as is well known, are synchronous with the periods of deflation of the lungs. The respiratory movements were registered by recording the movements of the larynx. The vagi above the heart were now carefully dissected, and all their branches between the

lower cervical ganglia and the roots of the lungs were cut. In some experiments the stellate ganglion and the thoracic sympathetic chain were also extirpated on both sides. The cardio-aortic innervation was thus destroyed, and the completeness of the destruction was shown by the disappearance of the vagus restraint of the heart. Moreover, it could be tested by an electric stimulation of the cervical vagus which, even when strong currents were used, produced no change whatever in the heart rate. It was found in these experiments that the section of the cardio-aortic fibres did not lead to the classical slowing down of the respiration. If, however, in these animals the main vagus trunks were cut in the neck, the slow vagal type of respiration made an immediate appearance.

It might possibly be objected that the animal was not in a normal condition because its respiratory movements were governed by the artificial ventilation of the lungs. In order to meet this objection, we modified the experiment in the following way. The right vagus was cut in the neck, the chest was opened, under artificial ventilation, through the second and third intercostal spaces on the left side (the third rib was left intact). By separating the ribs by means of retractors, the opening could be made sufficiently wide to allow a complete dissection of the left vagus, all the cardio-aortic branches of which were now severed. The completeness of the destruction of the cardio-aortic fibres was again tested by the stimulation of the intact vagus (left) in the neck; in no case was any change observed in the heart rate. After the end of the dissection, the chest wall was closed by suturing the intercostals and other thoracic muscles and the skin. The lungs were inflated in order to expel the air from the pleural cavity just before finally closing the wound. Artificial ventilation was then discontinued and the animal was kept under observation for over an hour. During the whole of this time, the respiratory movements of the animal showed no evidence of slowing down. Section of the left cervical vagus (the right nerve having been previously cut) led to an immediate development of the slow vagal type of respiration, although the cardio-aortic fibres had already been destroyed. This was always verified by dissection at the end of the experiment.

The following are the actual respiratory rates observed in one particular experiment. Before the section of the right cervical vagus, the rate of respiration was 26 per minute. After the section of the right nerve the rate slowed down to 18–20 per minute. After the section of the cardio-aortic fibres on the left side and after re-establishing the natural respiration, the respiratory movements showed some variations for a few minutes after the removal of the artificial ventilation, and then

settled down to the same rate of 18–20 per minute. This rate remained unchanged for about an hour. Cutting the left cervical vagus abruptly reduced the respiration to 10–11 per minute. The respiration remained slow and deep until the end of the experiment (over 4 hours). An actua record taken from a slightly modified experiment is reproduced in Fig. 2.

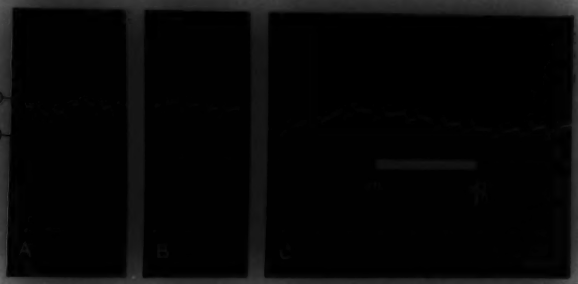

Fig. 2. Dog 10 kg. Chloralose. Stethogram and carotid blood-pressure. *A*. Record taken, 1 hour after the destruction of the cardio-aortic nerves on the left side. Respiration = 27 per minute. *B*. Record taken 45 min. after section of the right vagus in the neck; respiration = 22·5 per minute. The cardio-aortic innervation has thus been destroyed. In spite of this, the section of the left cervical vagus caused an immediate slowing of the respiration to 12–13 per minute and remained at this slow rate to the end of the experiment. *C*. Record taken 3 hours after section of the left cervical vagus. The respiratory rate = 13 per minute. The completeness of the cardio-aortic denervation is shown by the absence of the cardiac slowing during strong stimulation of the peripheral end of the cut left cervical vagus (stimulation is indicated by the signal).

The results of these experiments lead us to the conclusion that the cardio-aortic innervation cannot be responsible for the "respiratory vagal tone." Thus our experiments do not support the view advanced by J. F. and C. Heymans[1].

[1] In the discussion which followed the communication of this research to the XIVth International Congress in Rome, Prof. C. Heymans stated that his experiments led him to accept the pulmonary origin of the respiratory vagal tone. He does not, however, consider that this tone is entirely determined by the sensory impulses arising in the lungs. In part he believes it to be due to cardio-aortic impulses. We are unable to state whether, under some special conditions, impulses may arise in the cardio-aortic region which may modify the rate of the respiration. We cannot, however, find any evidence that cardio-aortic impulses invariably exert on the rate of the respiration a regulating influence which can be compared with the definite and constant dependence of the respiratory rate on the pulmonary innervation.

The pulmonary theory of the respiratory slowing down.

It is obvious that the most decisive proof for or against the pulmonary origin of the "respiratory vagal tone" would be provided by an experiment in which both lungs are completely denervated while all the other vagal fibres are left intact. This experiment was attempted by Boothby and Shamoff with a negative result. As mentioned above, they did not observe any change in the rate of respiration of their animals. Working aseptically on dogs, Boothby and Shamoff cut the branches of the vagi given off between the recurrent laryngeal nerves and the point where each vagus divides into its two primary gastro-intestinal branches. They claim that in this way all pulmonary and possibly a few cardiac branches are divided. Anyone who attempts this operation, however, will find that it is extremely difficult to make sure that all the pulmonary fibres have really been severed. It seemed to us that the denervation of the lungs could be carried out more simply and with a greater degree of certainty by the method which Anrep, Pascual and Rössler [1932] used in their experiments on cardiac arrhythmia. Thus, taking advantage of the anatomical distribution of the vagal fibres, it is possible to determine the dependence of the respiratory rate on the pulmonary innervation, by comparing the effects of section of the main trunks of the vagi just below and just above the roots of the lungs. Anrep and Segall [1926] showed that the section of the vagi just above the hila of the lungs does not disturb the cardio-aortic innervation. Indeed a careful dissection at the root of the lung shows that, besides a few œsophageal branches, all the other vagal branches in this region go to the lungs.

Section of the vagi below the roots of the lungs.

The chest wall was opened through the fifth intercostal space on the left side, the lungs being ventilated by the constant insuflation method of Meltzer and Auer. C. F. Palmer's small oscillating respiration pump was used for this purpose. The wound was widened by means of self-retaining retractors, and the branches of the vagi just below the hila of the lungs were exposed on the œsophagus. The nerves were surrounded by wires of two electro-cauteries, which were well protected so as not to touch any adjacent tissues. In some experiments sharp wire snares were used instead of electro-cauteries. The snares were arranged in such a way that they would cut through the nerves without displacing them by the traction. In experiments in which the nerves were to be stimulated,

shielded electrodes were used. The chest wall was then closed and the insufflation of the lungs was discontinued. The animals were allowed to recover for 1 or 2 hours, after which the vagi were cut by the snare or the electro-cautery. During this operation the chest remained closed.

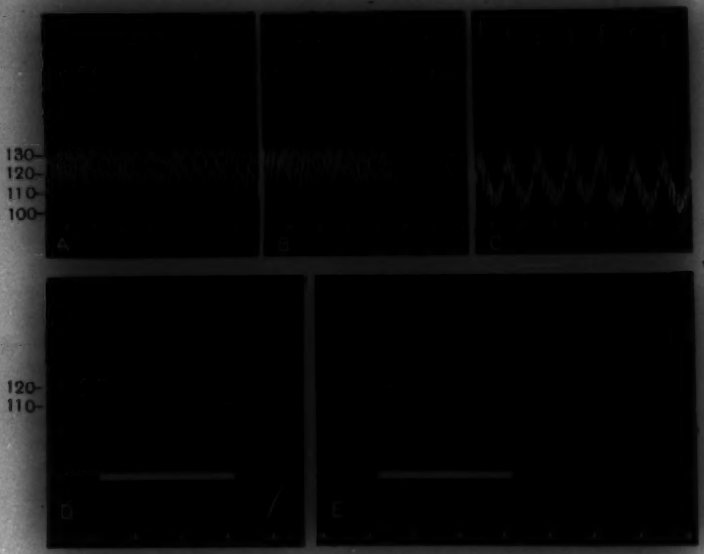

Fig. 3. Dog 6 kg. Sodium luminal. Stethogram and carotid blood pressure. *A*. Normal rate of respiration = 30 to 32 per minute. *B*. Record taken 20 min. after the section of the vagi below the roots of the lungs. Respiration = 32 per minute. *C*. Record taken 30 min. after section of the vagi just above the roots of the lungs. Respiration = 11·3 per minute. Records *D* and *E* are taken from another experiment. Dog 7 kg. Chloralose. *D*. Shows the effect on respiration of stimulation of the central end of the right cut vagus just below the root of the lung. *E*. Shows the effect on respiration of stimulation applied to the same nerve just above the root of the lung. The strength and the length of application of the stimulus were the same in both cases.

The section of the vagal trunks just below the hila of the lungs had no effect on the respiratory rate (Fig. 3 *A* and *B*). Electric excitation of the central ends of the cut nerves even with very strong currents also had no effect (Fig. 3 *D*).

In a few experiments, in order to be quite certain that all the vagal branches below the lungs were destroyed, we made a somewhat wider incision in the chest wall and, besides cutting the vagi as before, we also cut the œsophagus between the two ligatures. This, however, did not alter the result of the experiment. In every case the completeness of the section of the vagi was verified by dissection.

In three dogs the vagi were cut aseptically just below the hila of the lungs, and the animals were kept alive for 5 months. These animals showed no change in rate or depth of their respiration.

Section of the vagi above the roots of the lung.

The same methods were used for the section of the vagi just above the roots of the lungs. On the left side the vagus was approached through the third, and on the right side through the fourth intercostal space. The electro-cautery, the snare, or the shielded electrodes were placed round each nerve well below the cardio-aortic branches, at the point where it disappears behind the hilus of the lung. Special care was taken not to include the inferior laryngeal nerve. Four animals were operated upon aseptically and kept alive for a few days. Section of the vagi just above the hila of the lungs, performed after a preliminary section just below the hila, shows the effect of pulmonary denervation.

It was found in every experiment (48 dogs) that section of the vagi above the hila produced permanent slowing down of the respiration (Fig. 3 *C*). Electric excitation of the central ends of the cut nerves, even with weak currents, causes an immediate arrest of the respiratory movements (Fig. 3 *E*).

The fate of the operated animals will be discussed in another communication. It should be mentioned, however, that during the first few days of their survival (7–9 days) the respiration retained its typical slow character (5–9 per minute). The effect of section of the vagi above the hila was observed with different forms of anæsthesia. Ether is the worst type of anæsthetic for these experiments. In ether anæsthesia the respiratory centre is unduly excited, and section of the vagi just above the hila produces a considerably smaller effect than usual. In this we confirm the observations of M'Dowall [1927] and of Trevan and Boock [1922].

In a number of experiments, instead of cutting the vagi above the roots of the lungs, we tried to make an exact reproduction of the operation of Boothby and Shamoff; that is, the pulmonary branches them-

selves were cut, while the thoracic vagal trunk was left intact. These experiments were performed as follows. The left vago-sympathetic was cut in the neck, the chest was opened and the hilus of the right lung was exposed. The number of the branches going from the vagus nerve to the lung varies in different animals; usually there are about 5–7 separate

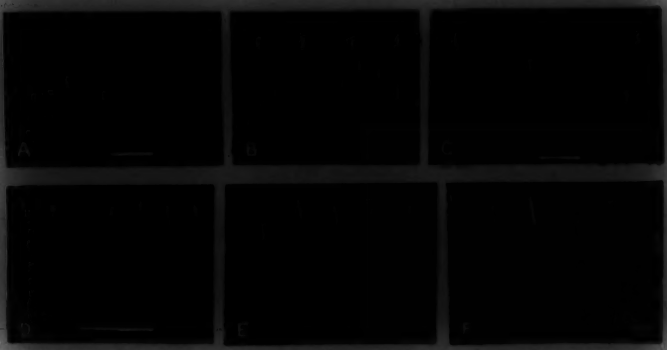

Fig. 4. Dog 6 kg. Chloralose. The respiration is recorded by registering the movements of the larynx. *A*. 40 min. after the section of the left vago-sympathetic nerve in the neck. Respiration = 32 per minute. An inflation of the lungs, indicated in the tracing by the white line, caused an arrest of the respiratory movements. *B*. 10 min. after section of the lower pulmonary branches of the right vagus at the root of the lung. Respiration = 32 to 36 per minute. The effect of inflation was unchanged. *C*. 10 min. after section of the middle pulmonary branches of the right vagus. Respiration = 23 per minute. Hering-Breuer's reflex is diminished in strength. *D*. 10 min. after section of the upper pulmonary branches. Respiration = 18·5 per minute. Hering-Breuer's reflex is absent. *E*. 15 min. after section of the smaller pulmonary filaments. Respiration = 13 to 15 per minute. *F*. The result of section of the right vago-sympathetic nerve in the neck. Respiration rate in the reproduced tracing = 13·5 per minute. The respiration actually fluctuated between 13 and 15 per minute. The rectal temperature of this animal was 39·8° C. which accounts for the slightly higher respiratory rate than that usually observed in the other experiments. The room temperature was 35·5° C.

branches. One or two big branches lie covered by the azygos vein, and can only be found after displacing the vein, or preferably after cutting it between two ligatures. Besides these branches there is a considerable number of very fine nerve filaments running from the vagus nerve to the lung. The pulmonary branches were cut in three stages starting from below upwards. After this the vagus trunk was completely separated from

the lung, to destroy the finer filaments. The completeness of the denervation of the lung was tested by cutting the vagus trunk above the pulmonary hilus. After each section the lungs were inflated in order to test the Hering-Breuer reflex. Fig. 4 shows the results obtained in one experiment. It can be noticed that the respiration begins to slow down only after at least two-thirds of the pulmonary branches were severed. The Hering-Breuer reflex at this stage is still present, but reduced in strength. After section of all the pulmonary branches, the respiration slows down still further and the Hering-Breuer reflex disappears. The lungs, however, still continue to exert an effect on the respiratory rate, as shown by the fact that the respiration shows a further slowing down after a complete separation of the vagus trunk from the lung. Section of the vagus trunk above the root of the lung, or in the neck, now completely fails to produce any change in the respiratory movements.

These experiments were repeated on animals whose chests were closed to avoid the complications introduced by the artificial ventilation of the lungs. The chest was opened on the left side, and the vagus trunk was completely separated from the hilus of the lung. After closing the chest, the animal was allowed to recover for an hour or two. Thus the laryngeal and the cardio-aortic innervation was preserved on both sides, while the pulmonary innervation was left intact on the right side only. The respiratory rate in one such experiment was 19–20 per minute. A complete denervation of the lungs was now made by cutting the right vagus in the neck. This reduced the respiratory rate to 10–11 per minute. A section of the left cervical vagus produced no further change in the respiratory rate. In other words, this section did not augment the effect of the denervation of the lungs, which would be the case if the "respiratory vagal tone" were even in part due to sensory impulses of cardio-aortic origin.

The comparison of the effect of section of the vagi below and above the roots of the lungs demonstrates the extent to which the classical changes in the respiration are produced by the denervation of the lungs. The following experiment shows that the pulmonary denervation entirely accounts for these changes, and that the destruction of other sensory fibres of the vagi does not assist in the production of the vagal type of respiration. Both nerves were cut just below the hila, electrocauteries were placed around the vagi immediately above the hila, and the cervical vagi were exposed in the neck. As mentioned before, section of the nerves below the hila had no effect on respiration. Section immediately above the lungs reduced the rate of respiration from 24 to

7 per minute. Both cervical vagi were cut 1 hour later, without producing any further change in the respiratory rate, which remained at 7–8 per minute until the end of the experiment (see Figs. 5 *A* and *B*, which are taken from another experiment). This observation is additional evidence against the cardio-aortic theory of Heymans, as well as against the laryngeal theory of Sharpey-Schafer.

The classical slowing down of respiration is not only produced by section of the vagi, but also by blocking the nerves by means of a constant current, or by the injection of novocaine (2 p.c.) into the trunks of the nerves. The block so produced is obviously only temporary; the effect of novocaine lasts for about an hour, and depends on the concentration and

Fig. 5. Dog 7 kg. Chloralose. Stethogram and blood-pressure. *A*. One hour after the bilateral section of the vagi just above the roots of the lungs. Respiration = 9 to 10 per minute. *B*. One hour after the section of both cervical vagosympathetic nerves in the neck. Respiration = 9 to 10 per minute.

the amount of solution injected. Blocking the nervous impulses in the vagi immediately above the roots of the lung produces the same effect as their section. Section of the cervical vagi, after their novocainization, just above the lungs fails to produce any further slowing down.

Bilateral intrathoracic section of the pulmonary branches of the vagi, performed aseptically with the view of keeping the dog alive for a long period of time, is an operation not easy to perform. We believe that the negative results obtained by Boothby and Shamoff are due to their having missed some of the fibres. This is especially likely because Boothby and Berry obtained a positive Hering-Breuer response in these animals. The observations described here show that, if a small number of the pulmonary fibres remain intact, this reflex can still be obtained.

SUMMARY.

1. The pulmonary, laryngeal and the cardio-aortic theories of the respiratory slowing down which follows double cervical vagotomy have been subjected to an experimental test.

2. The experiments fail to support Sharpey-Schafer's view that the classical respiratory slowing down is due to the paralysis of the laryngeal muscles.

3. They also fail to support Heymans' view that it is due to a cardio-aortic denervation.

4. The effect of double vagotomy on respiration is due to the denervation of the lungs.

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OBSERVATIONS ON PANTING.

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The first systematic investigation of the peculiar form of heat regulation by panting, observed in dogs, was made by Richet [1898]. As regards the mechanism of its action, Richet divides panting into reflex and central. Reflex panting is observed only in non-anæsthetized dogs, and is not necessarily accompanied by a rise in body temperature. When a dog is exposed to the sun or to heat, panting occurs within a few minutes, although the rectal temperature does not rise and may even drop below the prepanting level. Anæsthesia completely abolishes the reflex type of panting. In anæsthetized dogs, panting can only be obtained by raising the body temperature. When an anæsthetized dog is exposed to heat, the respiration begins to accelerate very gradually until the rectal temperature reaches 41·7° C., at which point the animal begins to pant. According to Richet, the reflex type of panting is due to a reflex excitation of the brain centres in response to the application of warmth to the skin and to the terminations of the fifth nerve, while the central type is due to direct stimulation by heat. Since Richet's work there has been no other systematic investigation of panting, although a good deal of research has been done on the changes which take place in the animal in response to raising the environmental temperature. In most of these researches, however, panting is discussed only as a secondary phenomenon. Bazett [1927] gives a detailed review and most of the literature on the subject.

The present investigation deals chiefly with that form of panting which Richet calls central, *i.e.* panting which is produced by raising the body temperature in anæsthetized dogs. The object of the experiments described here was to investigate the various changes taking place in the animal during panting and to study their interrelation.

Technique.

The majority of the experiments were performed on dogs weighing from 4 to 8 kg. The animals were anæsthetized either with urethane or, after administration of a small dose of morphine (5 mg. per kg.), with chloralose. Urethane and chloralose were injected intravenously, the first in doses from 0·25 to 0·5 g. per kg., the second in doses from 0·03 to 0·07 g. per kg., according to the depth of anæsthesia desired. Cats were used in a few experiments for the purpose of comparison. They were anæsthetized with urethane. The warming of the animals was carried out in a suitably arranged box, inside which were placed several carbon filament lamps. These lamps were completely shielded with metal screens. The temperature of the air inside the box could be raised to 50° C. or more within a few minutes. By extinguishing some of the lamps, the temperature of the box could be maintained at any desired level for an indefinite length of time. The animal was placed on thick pads of cotton-wool in the grooved bottom of the box, and consequently it did not touch any hot parts. The rectal temperature was measured throughout the experiment by a sensitive mercury thermometer, which was accurate to 0·1° C. The thermometer was introduced deeply into the rectum and was observed through a small opening in the box. The head of the animal protruded from the box and was supported by a holder.

The rate of respiration was registered by means of a stethograph and recorded on smoked paper or photographically. The total ventilation was recorded by a large Krogh's spirometer of 3·0 litres capacity, into which the animal expired through an outlet Douglas' or preferably Boulitte's valve, which was directly connected with a T-shaped tracheal cannula. The animal inspired air through an inlet valve on the other side of the cannula. The tracheal cannula was inserted as low down as possible so that the total dead space would be of the same order as that of the animal under normal conditions. The oxygen consumption was recorded by making the animal breathe through the same valves into a closed system of 22 litres capacity. The outlet tube of the trachea was provided with a carbon dioxide absorber (Siebe Gorman) and a small Krogh's spirometer of 700 c.c. capacity to record the respirations. Of the 22 litres of air in the system not more than 200 c.c. were allowed to be consumed at a time, after which the oxygen was replaced. The maximum drop of the oxygen concentration in the system was thus inappreciable.

The depth of respiration (stroke) was either calculated from synchronous records of the ventilation and of the respiration rate, or it was

registered directly by means of a Brodie's bellows recorder in a closed system of two Woulfe's bottles of 10 litres capacity, connected together by wide rubber tubing. The animal inspired from one bottle and expired into the other bottle, the carbon dioxide absorber being omitted. This method has the disadvantage of not being suitable for long observations, as the oxygen is used up and the carbon dioxide accumulates in the system. For this reason the bottles had to be renewed at short intervals, and only short records were taken. On the other hand, this method has the advantage of directly registering even negligible variations of individual strokes which can only be estimated roughly by the first method.

For analysis of the blood, samples were drawn under paraffin from the carotid artery. The carbon dioxide content was determined with van Slyke's constant pressure apparatus, while the oxygen saturation was determined with Haldane's blood-gas apparatus.

The temperature limits and the rate of panting.

On exposing an anæsthetized dog to heat, its rectal temperature rises slowly and its respiration accelerates more or less in relation to the rise of body temperature. At a certain temperature, however, this relation breaks and the respiration suddenly accelerates much further, although the temperature may not rise any more. Richet found that this abrupt change in the respiration took place always at 41·7° C. But the temperature of the onset of panting is found to be very dependent on the degree of anæsthesia. With light chloralose anæsthesia it develops at 39–40° C., with deeper anæsthesia it develops at 40·5–42° C., while in very deeply anæsthetized dogs panting may not develop at all, even with a rectal temperature of 42–43° C. An increase in the depth of anæsthesia in a panting dog abolishes panting, and it can only be re-established by a further rise of body temperature. When the animal is cooled, panting disappears if the rectal temperature falls by about 0·5° C. in deep anæsthesia, and sometimes by over 1·0° C. in light anæsthesia below the temperature of the onset of panting. Frequently, in lightly anæsthetized dogs, panting continues until the temperature is normal. The rate of panting depends on the depth of anæsthesia as well as on the temperature of the body. In light anæsthesia the usual rate of panting is 200–350 per minute, while in deep anæsthesia the rate may be as low as 120 per minute. With a further rise of temperature, the rate accelerates to the extreme which the anæsthetic can allow. It is frequently observed that in lightly anæsthetized animals the panting rate assumes periodic varia-

tions. Groups of fast and slow rates follow one another, sometimes with surprising regularity. These changes are generally, but not invariably, accompanied by changes in the stroke. The periods of fast rates may be of the same increased or diminished stroke.

We were able to confirm Richet's observation that panting is unchanged by the section of the cervical vagi. We found that the temperature limits and the maximum rate of panting are the same in vagotomized and in normal animals, if the depth of anæsthesia is controlled. It was suggested by Uyeno [1923] that, after section of the vagi, panting develops at a lower temperature and that the maximal rate obtained is lower than with the vagi intact. This may frequently be the case, but, since the onset of panting depends so much on the depth of anæsthesia, these results are inconclusive. We have on record many observations in which, at the same body temperature, panting reached a considerably higher rate after double vagotomy. If the anæsthesia is well controlled, we find no difference in the temperature of onset. Table I shows two observations on a 4·5 kg. dog which was given 0·3 g. chloralose, warmed and then cooled, with the cervical vagi intact. The vagi were then cut and the observation was repeated. During the second half of the experiment the animal was less under the effect of the anæsthetic. The rate of warming was the same in both cases.

TABLE I.

Rectal temp. °C.	Respiratory rate per min.	
	Vagi intact	Vagi cut
38·0	18	5
40·0	26	6
40·9	80–130	10–130–230
41·2	250	360
40·0	36	6
38·0	16	4

This experiment shows that, although the anæsthesia was somewhat less in the second case, panting developed at the same temperature and the rate after vagotomy was considerably higher than before. That the vagi do not play any rôle in panting is definitely shown by the fact that the respiration rate remains unchanged if the section of the vagi is made during panting (see Fig. 1).

In dogs with the vagi cut, the onset of panting is extremely abrupt. With the rise of rectal temperature, the slow and deep respirations of 4–8 per minute accelerate at first very slowly to 20–30, but when the panting temperature is reached the respiration jumps in a few seconds

to 120–150 per minute and assumes the characteristic shallowness of panting. The recovery from panting is also very abrupt, the shallow fast respirations suddenly becoming slow and deep as soon as panting disappears. The periodic variations in the rate and stroke are also observed after double vagotomy.

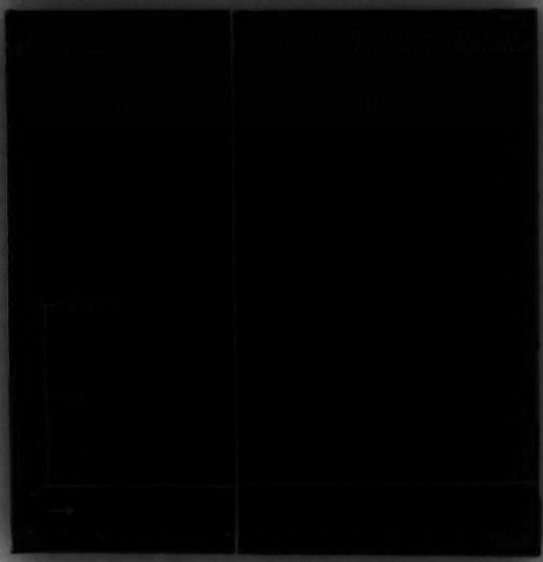

Fig. 1. The top record is the respiratory rate recorded by a stethograph, and the ascending undulating line is a record of the total ventilation. Time in seconds. Panting at 40·2° C. rectal temperature. Both cervical vagi were cut between records *A* and *B*. The interval between the two records was 30 sec. The respiratory rate was 290 and the ventilation 10,000 c.c. per minute in both records.

The stroke and the pulmonary ventilation.

Contrary to the opinion of previous authors, who regarded panting as a form of dyspnœa, Richet showed that panting is "a facilitated form of shallow and fast respiration." Other workers have confirmed the observation that the panting stroke is essentially shallow. In fact, normal dogs exposed to the sun always pant with a very shallow stroke,

unless the body temperature rises to an appreciable extent. On the other hand, when panting is caused by severe exercise such as running, the panting stroke is markedly deep. In anæsthetized dogs the changes in the stroke take a characteristic course. Between subnormal and normal temperature the stroke gradually increases; between normal and the panting temperature the stroke diminishes as the rate of respiration increases, but the diminution is small. As soon as panting sets in, however, the stroke rapidly diminishes to half the original or even less. In mild panting the stroke remains shallow; it returns to its original volume only after recovery from panting. In severe panting the stroke at first also becomes shallow, as in mild panting, but it gradually increases again to the original volume and may even considerably exceed it. On recovery from severe panting the stroke retraces the same course, becoming shallow at first and then gradually increasing to the original volume. Table II gives an example of these changes in the stroke.

TABLE II.

Warming				Cooling			
Rectal temp. °C.	Rate per min.	Stroke in c.c.	Ventilation in c.c. per min.	Rectal temp. °C.	Rate per min.	Stroke in c.c.	Ventilation in c.c. per min.
37·0	25	38	960	41·0	200	67	13,300
37·5	27	42	1,130	40·5	200	60	12,000
38·0	30	43	1,290	40·0	190	54	10,200
38·5	44	37	1,630	39·5	190	37	7,000
39·0	120	25	3,000	39·0	190	28	5,400
39·5	120	28	3,360	38·5	160	28	4,500
40·0	160	45	7,200	38·0	100	26	2,600
40·5	180	81	14,600	37·5	30	40	1,200
41·0	190	99	18,800	37·0	18	45	820
41·5	210	86	18,060				

The increase in stroke observed in severe panting is due to the rise of the animal's temperature after panting has already been established. If, after the onset of panting, the body temperature is not raised any further but kept strictly constant, the stroke remains small. Usually, however, in prolonged panting in anæsthetized animals, the stroke increases as panting continues. In this respect it is difficult to imitate the continued shallow type of panting obtained in dogs which, when exposed to the sun, pant at a normal body temperature, keeping up a fast rate of 300 per minute or more without increasing the stroke.

The ventilation during mild panting, in spite of the shallow stroke, increases 6–7 times above normal with rates of 120–150 per minute. In severe panting which is accompanied by an increase in stroke, the ventilation may increase 10–26 times with rates of 200–350. Frequently

it can be noticed, especially in severe panting, that groups of large and small strokes alternate with or without changes in rate. The most frequent case is a combination of fast rates with large strokes alternating with slower rates with small strokes, the ventilation obviously following the product of the change in stroke and rate. Section of the vagi in the neck performed during panting does not change the depth of the respiration. Since the respiratory panting rate is not affected by double vagotomy, the total ventilation obviously also remains unchanged (see Fig. 1).

In similar experiments on cats under urethane, Uyeno [1923], working in Cambridge, found that the highest rate of panting did not exceed 250 per minute and that "this change is not associated with a corresponding change in the total ventilation per minute which so far from being increased twelve or fifteenfold was never increased threefold, and on one occasion was not increased at all." In consequence of this, the respiration became very shallow, the observed respiratory depth being of the order of 2–3 c.c. or even less. As a result of this very shallow respiration the oxygen saturation of the arterial blood fell considerably below normal and the cats suffered from anoxæmia, which in some experiments attained a severe degree. In contradiction to Uyeno's experiments, we find in dogs that the total ventilation per minute increased to a considerably larger extent. We never came across a case of panting in which the ventilation was increased only threefold. Even in deeply anæsthetized dogs, if the animal panted at all, the ventilation increased not less than five to sixfold, the maximum increase observed being twenty-sevenfold.

In order to determine whether there is any difference between the dog and the cat in this respect, a number of experiments were performed on cats. No fundamental difference was found in the behaviour of the dog and the cat during panting. The following experiment may serve as an average example. A cat weighing 2·25 kg. was deeply anæsthetized with urethane and exposed to heat. The rate and ventilation were continuously recorded and the stroke was calculated. With a rectal temperature of 37° C. the rate was 24, the total ventilation was 480 c.c. per minute and the stroke was equal to 20 c.c. On raising the temperature of the animal to 39° C. the rate increased to 370, the ventilation to 3450 c.c. per minute and the stroke was equal to 9·3 c.c. The ventilation was thus increased over seven times. The increase in rate and ventilation was considerably greater than that observed by Uyeno, and was comparable with the result obtained in mild panting in dogs.

The considerable diminution of stroke recorded by Uyeno is due to the fact that in his experiments he used Müller's water valves, which do not give accurate registration of ventilation at such fast rates of respiration. Moreover, the whole aim of panting is to evaporate water from the respiratory passages. Making the animal breathe through water considerably reduces the chance of efficient evaporation.

The carbon dioxide content of the arterial blood.

Flinn and Scott [1923] found that, on exposing non-anæsthetized dogs to high temperatures, the carbon dioxide content of the blood drawn from the jugular vein diminishes, rapidly at first and then slowly. They believe that the fall in the carbon dioxide content of the venous blood is due to the severe hyperpnœa. Their records also show that the recovery of the carbon dioxide of the blood after the exposure is slow, being still incomplete even after an interval of 2 hours. Nasset and co-workers [1931] found a diminution in the carbon dioxide content of blood and plasma in the heat polypnœa caused by diathermy. The analysis of the arterial blood of panting dogs confirms these observations. Even in mild panting, in spite of the diminution of the stroke, the carbon dioxide content of the arterial blood may fall as much as 25–40 vol. p.c. in about 1 hour. In severe panting the secondary increase in stroke leads to a further diminution of the carbon dioxide content. In some experiments the carbon dioxide was reduced by 55–60 p.c. Table III shows

Table III.

Time in min.	Temp. °C.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	CO_2 content vol. p.c.	Lactic acid mg. p.c.
Exp. A						
0	38·0	20	750	37·5	46	27·7
50	39·9	32	1,060	33·1	43	31·5
90	40·5	240	5,000	20·8	28	39·7
110	40·5	270	6,750	25·0	28	42·2
160	37·8	34	1,000	29·4	36	27·7
200	37·5	24	912	38·0	38	27·0
Exp. B						
0	38·5	9	750	83·3	—	
10	39·0	9	820	91·1	46	
25	40·0	28	2,000	71·7	—	
35	40·0	50	3,600	72·0	44	
60	41·0	220	8,200	37·3	29	
90	41·2	250	18,000	72·0	22	

the results of two experiments in which the carbon dioxide content of the arterial blood happened to be the same before the onset of panting. The second animal was, if anything, more deeply anæsthetized than the

first. The difference between the two experiments was that in the first dog panting was severe, as shown by the considerable increase in the stroke. In the first case the carbon dioxide dropped in 90 min. by 39 p.c.; in the second, during the same interval of time, it dropped by nearly 55 p.c.

In the case of recovery from severe panting, the carbon dioxide content of the blood rapidly rises, with the diminution of the stroke observed during the transition from the severe to the mild form of panting, after which the recovery is slow. These changes in the carbon content of the blood are shown in Table IV. In this experiment, between the 107th and 117th min., the carbon dioxide rose from 23 to 33 p.c., as a result of the big diminution in stroke and total ventilation.

TABLE IV.

Time in min.	Temp. °C.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	CO_2 vol. p.c.
0	38·0	10	530	53·0	51
20	38·9	9	600	66·7	51
40	39·5	11	680	61·8	51
55	40·5	60	1,960	32·6	49
60	40·5	140	3,750	26·1	—
70	40·5	150	5,300	35·3	40
80	41·0	240	10,620	44·3	26
107	40·0	270	12,700	47·0	23
117	39·5	190	3,600	19·0	33
140	39·0	100	1,850	18·5	41
200	37·5	13	530	40·6	44
230	36·5	12	500	45·0	45

The relation between rate, ventilation, stroke and the carbon dioxide content of the arterial blood in mild and severe panting is shown in Fig. 2.

Anrep and Cannon [1923] found that over-ventilation of an animal leads to a considerable increase in the lactic acid concentration of the blood. They consider that this is due to alkalæmia, caused by the washing out of carbon dioxide. In view of this observation it was natural to enquire as to whether in panting the slow recovery of carbon dioxide might be due to lactic acid which had accumulated during panting.

In six experiments the lactic acid was determined in the arterial blood by Clausen's method, as used by Anrep and Cannon. In all these experiments the lactic acid was found to be somewhat increased, the maximum being reached during the period of the greatest over-ventilation. In the experiment reproduced in Table III the lactic acid concentration rose from 31·5 to 42·2 mg. p.c. In the other experiments

the increase was of the same order[1]. Flinn and Scott [1923] and Dadlez and Koskowski [1928] found no increase in lactic acid concentration in heat polypnœa.

In non-anæsthetized panting animals, in which the stroke appears to be very shallow, the washing out of the carbon dioxide is considerably

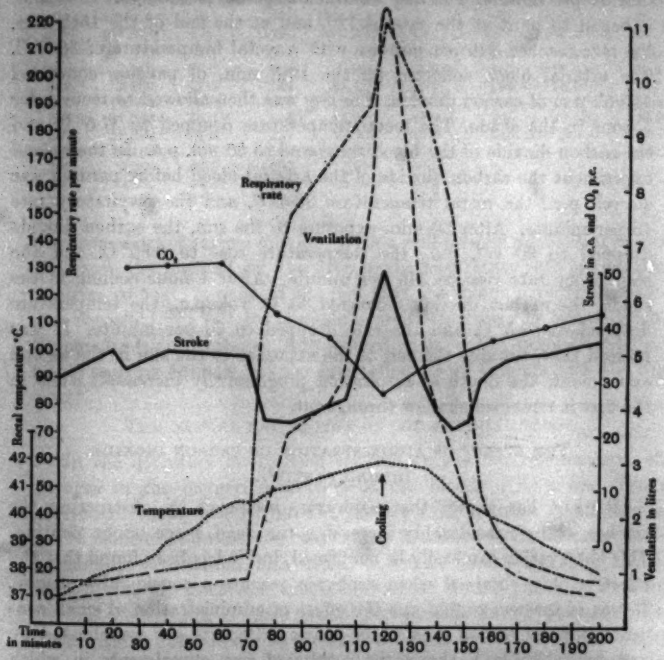

Fig. 2. The relation between the respiratory rate, ventilation, stroke and the CO_2 content of the arterial blood in panting produced by raising the body temperature.

less than in anæsthetized animals unless, as a result of long exposure to the sun, the body temperature rises appreciably and the depth of respiration increases. This is illustrated by the two following experiments. Under light anæsthesia with C. and E. mixture, a cannula was

[1] We wish to express our sincere thanks to Dr A. Hassan for having carried out the analyses of the blood samples for lactic acid.

introduced into the carotid artery, the dog was allowed to recover from the anæsthetic and left undisturbed for 2 hours. The first blood sample was then collected; the second sample was collected 50 min. later. The carbon dioxide contents were found to be 49 and 50 vol. p.c. respectively. In both cases the rectal temperature was 38·2° C. and the respiratory rate 30 per minute. The dog was then exposed to the sun. In 5 min. it began to pant at the rate of 120, and at the end of the 15th min. the rate reached 240 per minute, with a rectal temperature of 38·3° C. The arterial blood collected on the 16th min. of panting contained 44 vol. p.c. of carbon dioxide. The dog was then allowed to recover for 1 hour in the shade. The rectal temperature dropped to 37·5° C. and the carbon dioxide of the blood recovered to 50 vol. p.c. In the second experiment the carbon dioxide of the arterial blood before panting was 46 vol. p.c., the rectal temperature 38·5° C. and the respiratory rate 40 per minute. After 50 min. exposure to the sun, the carbon dioxide dropped to 30 vol. p.c., the temperature rose to 39·5° C. and the respiratory rate rose to 340 per minute. After 1 hour cooling in the shade, the carbon dioxide recovered to 44 vol. p.c., the temperature dropped to 38·5° C. and the rate dropped to 60 per minute. It was noticed that, towards the end of the exposure to the sun in the second experiment, the depth of respiration progressively increased, while in the first it remained shallow throughout.

The effect of administration of carbon dioxide during panting.

Richet has shown that asphyxia, produced by obstructing the trachea or by considerably increasing the dead space, stops panting. This observation can easily be confirmed, but it has been found that the effect is only obtained when asphyxia reaches a considerable degree. It was of interest to find out the effect of administration of small concentrations of carbon dioxide on panting which is not complicated by anoxæmia. Table V shows the results of two experiments in which different concentrations of carbon dioxide were administered.

Small concentrations of carbon dioxide in the inspired air definitely increase the depth of respiration but have little or no effect on the rate of respiration. The total ventilation is thus increased in proportion to the increase in the stroke. Somewhat higher concentrations of carbon dioxide lead to a greater increase in depth of respiration but also diminish the rate of panting. However, in spite of the diminution of the rate, the total ventilation is increased to a much larger extent than with small

TABLE V. The period of administration of carbon dioxide is shown by heavy type. Every figure is an average of several readings. The dog's temperature was 37° C. before panting and 41° C. during panting. Panting at 40° C.

Exp. A 2 p.c. CO_2 for 6 min.			Continuation of Exp. A 4 p.c. CO_2 for 5 min.			Exp. B 7·6 p.c. CO_2 for 2 min.		
Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.	Rate per min.	Ventilation in c.c. per min.	Stroke in c.c.
11	600	54·5	200	7,400	37·0	240	8,450	35·2
200	7,200	36·0	200	9,500	47·5	210	10,600	50·5
200	9,500	47·5	190	11,150	58·7	120	12,000	100·0
200	10,000	50·0	190	13,300	70·0	110	12,900	117·3
200	11,050	55·3	190	15,000	83·3	200	11,250	56·3
200	11,300	56·5	170	15,000	88·2	240	7,800	32·5
200	10,400	52·0	190	13,300	70·0			
200	9,250	46·3	200	12,600	63·0			
200	8,850	44·3	200	9,500	47·5			
200	7,400	37·0	200	710	35·3			

concentrations of carbon dioxide. With a still greater increase of carbon dioxide in the inspired air, the drop in the respiratory rate is very conspicuous so that, in spite of the fact that the stroke may be increased over threefold, the relative increase in ventilation may be less than with the smaller concentrations. Administration of carbon dioxide to vagotomized panting animals has the same effect as on animals with the vagi intact.

THE OXYGEN SATURATION OF THE ARTERIAL BLOOD.

In the literature on panting we find only a few references to the changes in the oxygen saturation of the blood. Flinn and Scott, analysing venous blood from the jugular vein, found a small increase in the percentage saturation, which they attribute to the increase of the pulmonary ventilation. This could, however, be explained by the increased rate of circulation which is observed during panting [Uyeno]. On the other hand, Uyeno found that in cats the oxygen saturation of the arterial blood falls as a result of a great reduction of the stroke. His panting animals actually suffered from severe anoxæmia, and showed in some cases a drop of oxygen saturation from 95 to 56 vol. p.c. As a rule the oxygen saturation during panting was found to be between 80 and 90 p.c. As mentioned before, Uyeno's results are most probably due to the fact that he used unsuitable valves, which did not allow of proper ventilation of the animal during the fast breathing. Experiments on panting dogs show that in no case, even in severe panting, is the oxygen saturation of the blood reduced, and this applies both to non-anæsthetized animals which are exposed to the sun and to anæsthetized

animals which are warmed in a box. Table VI shows the results of three experiments. The first was performed on a dog which was anæsthetized with a moderate dose of chloralose; the second was on a dog which was very deeply anæsthetized, and the third on a non-anæsthetized dog. The first two dogs were warmed in the box and the third was exposed to the sun.

TABLE VI.

Exp. A			Exp. B			Exp. C		
Temp. °C.	Rate per min.	Oxygen saturation p.c.	Temp. °C.	Rate per min.	Oxygen saturation p.c.	Temp. °C.	Rate per min.	Oxygen saturation p.c.
38·0	17	93·7	38·0	22	85·2	38·2	30	96·4
38·0	17	94·5	41·0	40	85·4	39·2	300	96·8
39·3	290	97·2	41·4	235	90·9	38·5	330	96·4
39·8	300	96·7	41·0	260	95·6	38·2	40	96·8
37·0	22	96·2	39·0	33	88·4	38·2	25	96·8
37·0	17	95·4	35·5	25	88·2			

Far from showing any anoxæmia, the lightly anæsthetized animal, whose oxygen saturation was somewhat lower than normal (probably on account of the anæsthesia), showed a small rise of the oxygen saturation. The oxygen saturation of the deeply anæsthetized animal greatly improved during panting, while in the non-anæsthetized animal the oxygen saturation remained unchanged. The oxygen capacity of the arterial blood is considerably increased in panting, a fact which is explained by previous observers as due to the considerable loss of water by the animal.

The experiments of Uyeno were performed on cats, and it seemed possible that, although dogs fail to show anoxæmia during panting, cats might behave differently. The following experiment (Table VII) shows

TABLE VII.

Time in min.	Temp. °C.	Rate per min.	Ventilation c.c. per min.	Stroke in c.c.	CO_2 vol. p.c.	O_2 saturation p.c.
0	Douglas' valves on					
15	39·8	260	2600	10·0	34	96·6
18	39·8	Müller's valves on				
20	40·0	260	802	3·1	—	—
35	—	240	1000	4·1	—	80·0
37	—	230	1120	4·8	43	—
38	—	240	1000	4·1	—	75·5
39	—	Müller's valves off, cat breathing without valves				
40	—	330	—	—	—	—
42	Douglas' valves on					
45	—	290	3110	10·7	—	—
60	—	320	3462	10·6	—	—
80	—	350	3350	9·6	—	98·0
90	—	330	3350	10·1	—	—
	Cooling					
120	37·5	40	650	16·3	—	—

that this is not the case. A cat weighing 2·5 kg. was anæsthetized with urethane. The total ventilation was measured in the same manner as in dogs, and the observations were made alternately with the cat breathing through Douglas' and through Müller's valves. When the anæsthetized cat was placed on the table, it was already panting, the room temperature being 34·0° C. and the rectal temperature 38·5° C. The observations began when the rectal temperature registered 39·8° C., which happened after 45 min. of warming. Douglas' valves were then connected with the tracheal cannula. The first sample of arterial blood was collected after the animal had breathed through these valves for 15 min.

This experiment illustrates the inefficiency of Müller's valves. When used during panting they lead to a condition of rebreathing. This is further supported by the fact that the arterial carbon dioxide content increased during the period of breathing through Müller's valves, which never happened when no valves or when Douglas' valves were used.

The effect of anoxæmia on panting.

Uyeno considered that the high rate of respiration obtained on exposing cats to heat is always accompanied by anoxæmia. Although our experiments show that this is not the case, it was of interest to investigate the extent to which panting is affected by anoxæmia and to compare the effect of anoxæmia with that of administration of carbon dioxide. Anoxæmia was obtained by allowing the animal to rebreathe in a closed system through a carbon dioxide absorber. The total ventilation and the rate of diminution of oxygen content of the closed system was measured graphically. The actual technique employed is shown in Fig. 3. Anoxæmia was continued until some definite change in the panting was observed, after which the dog was allowed to recover. The inspired air was not analysed, but arterial blood samples were taken at short intervals of time, and their oxygen saturation was determined. It was clear at once that the effect of anoxæmia on panting differs greatly from that of administration of carbon dioxide. Panting is not affected by anoxæmia until the oxygen saturation falls below about 80 p.c., the total ventilation and the respiratory rate remaining unchanged. As anoxæmia progresses further, the stroke gradually diminishes while the rate remains unchanged, the total ventilation obviously diminishing in proportion to the diminution in stroke. This change in depth of respiration becomes more and more conspicuous. With a further drop of oxygen saturation,

the stroke becomes progressively more and more irregular, shallow strokes alternating with periods of deeper strokes. The rate of respiration also begins to show variations, faster rates usually coinciding with deeper strokes. The rate, however, is never affected to the same extent as the

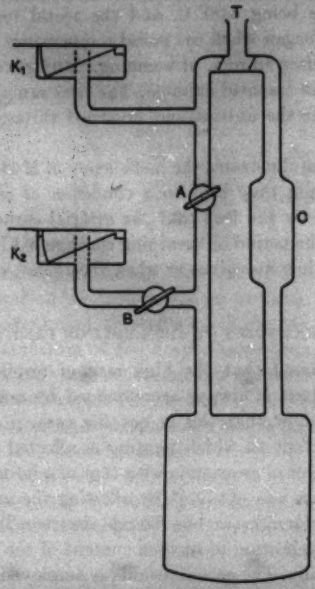

Fig. 3. Method used for determining the total ventilation of an animal breathing in a closed system. K_1 and K_2 are spirometers of 3 litres capacity each. The total capacity of the apparatus is about 15 litres. The ventilation is recorded by K_1 after closing the tap A. The animal inspires from K_2 and expires into K_1. The CO_2 is absorbed in C. On emptying spirometer K_2 and closing tap B, spirometer K_1 registers the O_2 consumption. T is a tracheal cannula with two Douglas' valves.

stroke. These irregularities gradually become more pronounced, until the oxygen saturation of the arterial blood drops below about 45–50 p.c., when the respiratory mechanism shows a complete breakdown. Panting rapidly disappears and is replaced by slow and deep respiration, frequently of the Cheyne Stokes character. When the animal recovers from anoxæmia, the changes in rate and stroke retrace their course, but the

periodic variations remain for a long time. A comparison of the effect of anoxæmia with that of administration of carbon dioxide is shown in Table VIII.

TABLE VIII. The total capacity of the apparatus is about 15 litres.

Time	Rate per min.	Ventilation c.c. per min.	Stroke in c.c. per min.	CO_2 content vol. p.c.	O_2 saturation p.c.	Amount of O_2 consumed
0	300	12,400	41·3	27	95·0	0
—	Rebreathing started					
10	300	12,000	40·0	—	—	—
15	290	10,540	36·3	31	74·9	1000
20	290	9,400	32·4	—	—	—
25	310	6,400	20·7	34	50·6	1700
30	The respiration became very irregular, panting disappeared					2000
	The animal was allowed to recover for 40 min.					
0	270	12,000	44·5	27	95·4	—
1	4·5 p.c. carbon dioxide administered to the animal					
3	260	12,000	49·6	—	—	—
8	210	15,200	72·4	—	—	—
10	210	16,000	76·0	—	—	—
12	195	15,500	79·5	—	87·4	—
15	140	14,000	100·0	44	—	—
16	Carbon dioxide turned off					
25	270	12,400	46·0	23	96·4	—

THE OXYGEN CONSUMPTION DURING PANTING.

On raising the environmental temperature, the total metabolism of the animal increases as the body temperature rises. Du Bois [1921] estimated that the Q_{10} factor in prolonged experiments on man is equal to 2·3. In short experiments, according to Landis and co-workers [1926] and Koehler [1923], the Q_{10} amounts to 6·9 and 12·1 respectively, and according to McConnell and Yagloglou [1925] to 31·0. These discrepancies are explained by Bazett as being due to the pronounced hyperpnœa which accompanies the rise of body temperature. Our experiments support this hypothesis and show that, apart from the increased metabolism of the whole body, there is a large increase of oxygen consumption during panting, which can be accounted for by the increased work of the respiratory muscles. With a rise of body temperature the oxygen consumption increases in two stages; (*a*) a gradual rise closely following the rise of the rectal temperature up to the point of the onset of panting. In a dog of 5·5 kg., deeply anæsthetized with chloralose, the oxygen consumption rose from 50 to 70 c.c. per minute with a rise of rectal temperature from 36·5° to 40·5° C., *i.e.* a rise of 40 p.c. for 4° C. (*b*) A further sharp rise with the onset of panting, which is proportional to its extent, and which subsides when the animal recovers from panting. The oxygen consumption of the above dog rose to 75 and later to 85 c.c.

per minute, with respiratory rates of 100 and 190 respectively, although the temperature rose only by 0·2° C. The change in oxygen consumption which takes place during panting is shown in Fig. 4 *A*.

The experiment reproduced in Fig. 4 *A* was performed on the second day after double cervical vagotomy, which accounts for the very slow respiratory rate before panting. The results obtained on dogs with intact vagi do not differ in any way from those obtained after vagotomy.

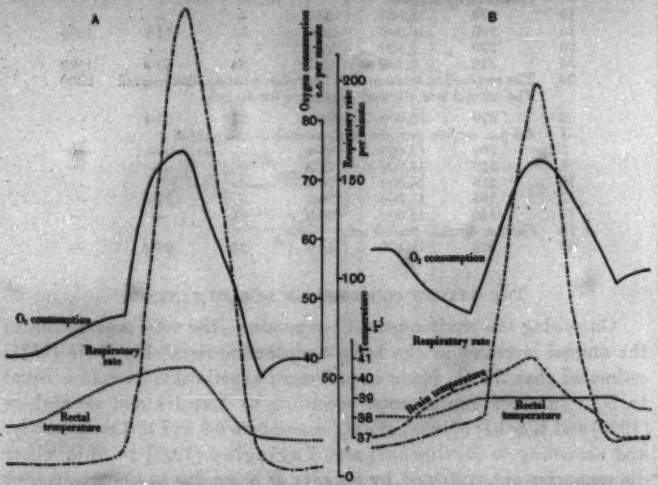

Fig. 4. The effect of panting on the O_2 consumption, (A) on warming the whole animal, (B) on warming the carotid blood. In A, the dog weighed 3·75 kg., and the experiment was made on the second day after double cervical vagotomy. In B, the dog weighed 6·5 kg., and both vertebral arteries were tied, and the carotid blood was passed through a glass spiral for the purpose of warming. The thermometer registering the temperature in the head was placed deeply under the tongue. The animal was heparinized.

In this type of experiment the body and the brain temperatures of the animal rise alike. In view of the abundant evidence in the literature that warming the brain leads to a diminution of the metabolism, the increase in oxygen consumption observed in panting, on warming the whole body, is obviously the result of several conflicting factors.

The increase of oxygen consumption in the case of panting presents,

however, no fundamental difference in animals in which the whole body is subjected to heat and animals in which panting is obtained by warming the carotid blood. The pre-panting stage, however, is different. In the first case, the oxygen consumption gradually rises with the rise of body temperature, while in the second it diminishes with the rise of brain temperature (see Fig. 4 *B*). Thus the abrupt increase of oxygen consumption, determined by the excessive activity of the respiratory muscles during panting, is in the first case accentuated by the increase in the general metabolism, while in the second case it is antagonized by the central regulating mechanism.

It has been shown by previous workers that panting does not take place after decerebration at the level of the anterior colliculi. The experiments of one of us (M. H.) confirm this observation. The respiratory rate never rises after decerebration beyond 60–70 per minute. In these animals the oxygen consumption follows the changes of the body temperature and does not show the abrupt increase characteristic of panting.

Summary.

1. Panting observed on raising the body temperature was studied in dogs and cats.

2. The body temperature at which panting begins depends on the depth of anæsthesia. In lightly anæsthetized animals, panting may start at a temperature which is only slightly above normal.

3. In mild panting, the stroke is considerably smaller than normal. In severe panting, the stroke increases to normal or above.

4. In mild panting, the total ventilation is increased from six to sevenfold. In severe panting, it may be increased as much as twenty-five to twenty-sevenfold.

5. In panting, the animal is over-ventilated. The CO_2 content of the arterial blood diminishes in mild panting by 25–40 p.c. and in severe panting by 55–60 p.c.

6. Our experiments fail to support Uyeno's statement that panting animals suffer from anoxæmia. The O_2 saturation of the arterial blood in panting dogs and cats is not diminished. On the contrary if, on account of deep anæsthesia, the O_2 saturation is below normal before the period of panting, it rises during panting.

7. Administration of CO_2 to a panting animal greatly increases the stroke and somewhat diminishes the rate of respiration. The total ventilation is increased.

8. Artificially induced anoxæmia has little effect on panting until the O_2 saturation of the arterial blood falls below 80 p.c. Between 80 and 50 p.c. of O_2 saturation, the rate is almost unchanged, while the stroke shows progressive diminution. At about 50 p.c. saturation, the "respiratory panting mechanism" is put out of action.

9. The O_2 consumption during panting is increased in proportion to the total ventilation.

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THE PRESSURE OF AQUEOUS VAPOUR IN THE ALVEOLAR AIR.

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The pressure of aqueous vapour of the alveolar air is generally accepted to be a physiological constant. In calculations of alveolar gas pressures the value 47 mm. Hg is used, a value which represents the pressure of aqueous vapour in air saturated with moisture at 37·2° C. To our knowledge no direct measurement of the pressure of water vapour in the alveolar air has ever been made, and even from theoretical considerations it would seem probable that the figure which has been accepted is too high. It would also seem possible that this value might readily show physiological variations. This is evident when we consider the assumptions made in assuming a vapour pressure of 47 mm.—It is assumed that (*a*) the osmotic pressure of the pulmonary epithelium is equal to that of the blood; (*b*) the alveolar air is always saturated with moisture; (*c*) the temperature of the alveolar air is 37·2° C. and does not vary. The validity of these assumptions will be discussed later.

Methods.

Various volumetric, gravimetric and dew-point methods were tried, but we have only found the two which are described to be satisfactory.

(*a*) *Dew-point method.*

To obtain an accurate measurement of the temperature at which condensation of the water vapour in the alveolar air takes place, we have found the simple arrangement shown in Fig. 1 entirely satisfactory. A 500 candle-power parallel beam of light (*A*) is made to traverse a 1 litre pyrex flask (*B*) filled with water, the temperature of which can be accurately read to ± 0·02° C. After traversing the flask the beam is reflected by a mirror (*C*) in such a way that it just misses a second mirror

(*D*) which is placed to one side of its path. The faintest trace of moisture condensing on the pyrex flask at the point of exit of the beam of light (*E*) will cause some scattering of the light rays, and some of these will impinge on the second mirror (*D*). This will show as a bright flash if the condensation be only transitory or as a steady glow if the condensation remains. The surface of the flask must be scrupulously clean (we have found that the use of concentrated nitric acid and distilled water is sufficient) and the water in the flask distilled. At the beginning of the experiment the water is heated to approximately 40° C. and then

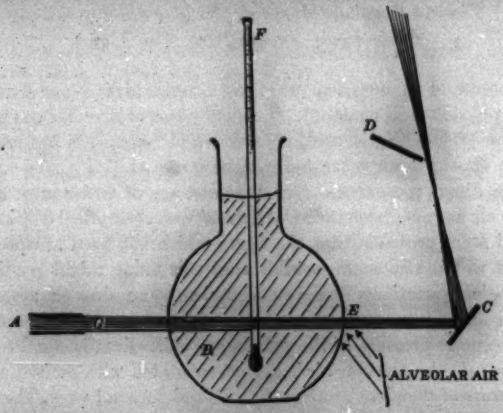

Fig. 1. Dew-point method. *A*, 500 candle-power light; *B*, pyrex flask filled with distilled water; *C*, mirror; *D*, mirror; *E*, clean surface of flask on which alveolar air is made to impinge; *F*, thermometer; *G*, parallel beam of light.

allowed to cool slowly, temperature equilibrium being ensured by continuous stirring with the thermometer. At frequent intervals samples of alveolar air are made to impinge on the pyrex flask at the point of exit of the beam of light. Whenever a flash of light is observed on the second mirror (*D*) a temperature reading is made. This temperature represents the dew point of alveolar air and from this the vapour pressure can be calculated directly. The alveolar air is made to impinge on the pyrex flask simply by making a forced expiration with the mouth close to the point of exit of the beam of light. The expiratory act should take about 3 sec., and with a little practice it is easy to stir the water, observe the mirror (*D*), and expire forcibly, at the same time.

With this system, successive measurements of the vapour pressure of the alveolar air will not vary by more than ± 0·2 mm. of Hg. To form some estimate of the absolute accuracy of the method we have measured the vapour pressure of air artificially saturated with moisture. Air is passed through a water pumice stone tower, its temperature is accurately measured, and it is then allowed to impinge on the pyrex flask. The temperature of the air was always found to be somewhat higher than the temperature of the flask at which condensation was observed (Fig. 2), the discrepancy amounting to an average of 0·23° C. in 57 observations. Unfortunately, since the evaporation of water produces an appreciable lowering of the temperature in the saturating chamber, it is impossible with this system to have complete temperature

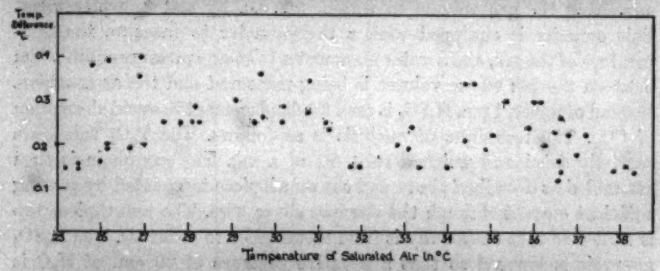

Fig. 2. Calibration of dew-point method. Showing difference between temperature of dew formation and temperature at which air was saturated with moisture.

equilibrium between the air saturated with moisture and its environment. For this reason we strongly suspect that some at least of the variations shown in Fig. 2 are due to unsaturation of the air caused by increase in temperature after saturation. This supposition is strengthened by the fact that a much smaller variation was obtained when air saturated with water vapour in the lungs was used. However, the factor 0·23° C. certainly represents the maximum difference between the true dew point and the observed dew point by this method, and as such has been used as a constant correction factor.

(*b*) *Gravimetric method.*

To measure the weight of water in a known volume of alveolar air we have passed successive Haldane-Priestley samples of alveolar air through a chain of P_2O_5 tubes and then carefully measured the total volume of alveolar air by water displacement.

A three-way glass tap of 4 mm. bore is wound with several yards of insulated German silver wire and the whole bound with asbestos string. By passing a 12-volt current through the wire the tap can be heated to well above body temperature. One limb of the tap is covered with rubber so as to serve as a mouthpiece and so placed that by turning the tap expiration may take place through one or other of the airways. One of these is left open to the atmosphere and the other directly connected to two large P_2O_5 tubes in series, filled with P_2O_5 and glass-wool. After having traversed these tubes the air is led through a gas sampling tube of 1 cm. bore and 25 cm. length, followed by a tower filled with pumice stone saturated with 1 p.c. sulphuric acid to saturate with water and avoid absorption of CO_2, and finally a graduated cylinder capable of measuring the gas volume to within $\pm$ 2 c.c. by water displacement. This cylinder is equipped with a thermometer to measure the temperature of the gas, and a water manometer to ensure pressure equilibrium between the gas whose volume is being measured and the atmosphere. Instead of water, 1 p.c. H_2SO_4 is used for displacement to avoid absorption of CO_2. The technique of analysis is as follows. The P_2O_5 tubes are carefully dried and weighed to $\pm$ 0·1 of a mg. The gas chain is then assembled as described above and the mouthpiece tap heated by passing sufficient current through the German silver wire. The mouthpiece tap is turned so that the airway is from mouthpiece to room air. The H_2SO_4 reservoir is lowered so that a negative pressure of 20 cm. of H_2O is developed in the system. Flow of water between the reservoir and the graduate cylinder is then prevented and, by observing the level in the water manometer, the presence of any leak detected. If no leak occurs the experiment is begun and a negative pressure of 20 cm. of H_2O throughout the system maintained. A deep breath is taken and the lips firmly applied to the mouthpiece on the three-way tap, which is heated to a temperature which can just be tolerated. The breath is held for 20 sec. and a slow expiration lasting from 3 to 5 sec. made, so as almost but not completely to expel the complementary and reserve air. The three-way tap is then turned so that from 100 to 150 c.c. of the air from the lungs are drawn through the gas chain. This procedure is repeated until 5 litres of alveolar air have been measured by the measuring cylinder. The P_2O_5 tubes are again weighed and the vapour pressure calculated from the usual formula $PV = \frac{W}{M}RT$. The following points are important. As the sample of alveolar air which is drawn through the gas chain remains in the mouth for several seconds, breathing must be through the nose during and prior to each deep breath. No saliva must

be allowed to touch the limb of the three-way tap in the mouthpiece. There must be no condensation of moisture in the arm of the P_2O_5 tube within 2 cm. of its termination.

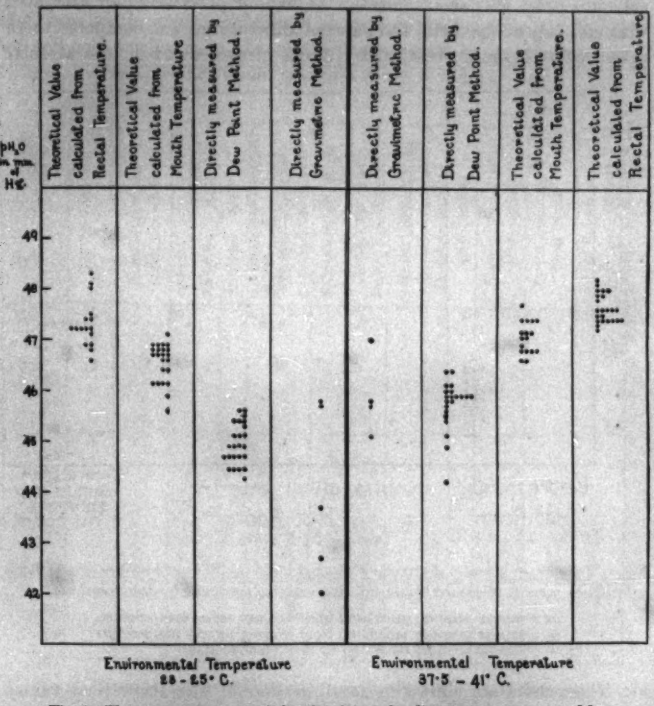

Fig. 3. The vapour pressure of the alveolar air by direct measurement and by calculation from the rectal and mouth temperatures.

In spite of the utmost care the figures obtained for the vapour pressure of the alveolar had a range of almost 3 mm. of Hg, which compares unfavourably with those obtained by the dew-point method (Fig. 3). Also an observation by this method takes 2 hours or more, while by the dew-point method only from 2 to 5 min. are required.

Results.

As might be expected the vapour pressure of the alveolar air was found to follow closely changes in the systemic temperature. For the sake of clarity we have calculated the vapour pressure of air saturated with moisture at the rectal and mouth temperatures, and compared these pressures with those obtained by direct measurement on the alveolar

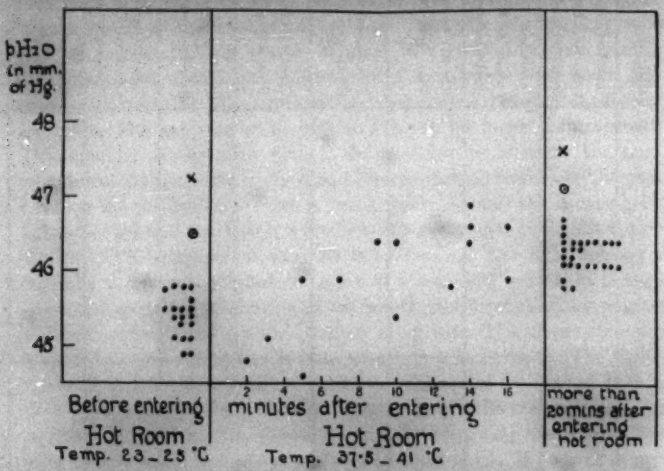

Fig. 4. The vapour pressure of alveolar air brought into equilibrium with the venous blood by 45 sec. apnœa. Measured before and after entering hot room, by dew-point method.

× = vapour pressure calculated from average rectal temperature.
⊙ = vapour pressure calculated from average mouth temperature.
• = vapour pressure measured by dew-point method.

air. The results are shown in detail in Fig. 3. The theoretical values calculated from the rectal and mouth temperatures average 47·26 and 46·55 mm. respectively, whereas the measured values average 44·91 mm. by the dew-point method and 44·0 by the gravimetric. When the temperature of the environment was raised to between 37·5 and 41° C. the vapour pressure calculated from the rectal and mouth temperatures rose to average values of 47·65 and 47·16 mm. respectively, followed by the alveolar air which rose to 45·81 mm. by the dew-point method and 45·9 by the gravimetric (Fig. 3).

Effect of voluntary apnœa.

When complete equilibration between blood and alveolar air was ensured by holding the breath for a period of 45 sec., the same change was found to take place, the vapour pressure rising from an average of 45·43 mm. in the cold environment to 46·26 in the hot (Fig. 4). In both hot and cold environment the apnœa produced *per se* an average rise of 0·5 mm. in the alveolar vapour pressure (Figs. 3 and 4).

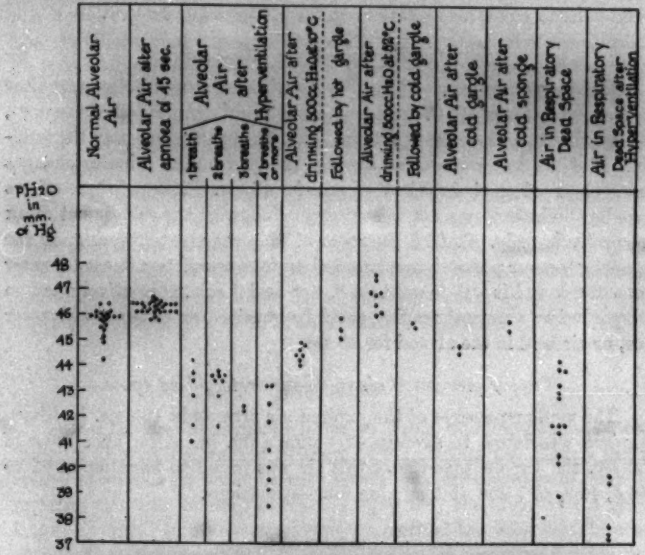

Fig. 5. Variations in the vapour pressure of the alveolar and dead space air. Dew-point method. Temperature of environment 37·5–41·0° C.

Effect of hyperventilation.

One full inspiration immediately followed by a forced expiration is sufficient to produce a perceptible lowering of the vapour pressure of the alveolar air as measured by the dew-point method (Fig. 5). The average values found were 42·8 mm. after one deep breath, 43·2 mm. after two breaths, 42·3 mm. after three breaths and 40·9 mm. after four

or more breaths. The lowest value obtained was 38·5 mm. after eight deep breaths. These experiments were all conducted in an environmental temperature of from 39 to 41° C.

Rapid and shallow breathing.

After voluntary tachypnœa of 45 sec. duration with a respiratory rate of 60 a minute and depth of approximately 200 c.c., the vapour pressure of the expired air at the end of such a shallow breath amounted to an average of 38·3 mm. (Fig. 5). These experiments were also conducted in an environmental temperature of from 39 to 41° C.

Effect of cooling mouth and naso-pharynx.

If the vapour pressure of the alveolar air is measured before and after 500 c.c. of water at 12° C. are drunk, a slight but definite fall is observed, amounting to an average of 1·3 mm. (Fig. 5). In the same way the imbibition of hot water causes an average rise of 1·0 mm. Similar changes were observed on air held in the lungs for 45 sec. Either of these effects can be eliminated by a hot or cold gargle following the cold or hot drink respectively (Fig. 5). The duration of this apparent lowering of the alveolar vapour pressure was followed on four occasions after cold water was drunk and it was found that 8, 8, 6 and 4 min. respectively had to elapse before a normal reading could be obtained on either the alveolar air, or air held in the alveoli for 45 sec.

Vapour pressure of air in the respiratory dead space.

The vapour pressure of the expired air after only 100 c.c. had been expired was found to average 42·1 mm. (Fig. 5). After an apnœa of 30 sec. the vapour pressure of this air was found to have mounted to 44·4, 44·9, 45·1 and 45·1 mm. on four estimations.

Criticisms.

These experiments, especially those with hot and cold water, immediately suggest that under any conditions, whether with quiet respiration or with hyperventilation, the alveolar air may be cooled during its passage from the alveoli to the mouth. Were this true any application of the dew point as measured by this method to the vapour pressure of the alveolar air would be entirely fallacious. One purpose of the experiments which have been described was to prove or disprove this possibility, and, when we analyse the evidence, it would seem to be entirely against such a supposition.

(*a*) Rapid and shallow breathing produces no lowering of the vapour pressure of the true alveolar air. Neither rapid and shallow breathing nor hyperventilation produced any lowering of the vapour pressure of air subsequently held in the lungs for 45 sec. With cold water, on the other hand, the lowering of the dead space temperature sufficient to produce a fall in vapour pressure of only 1 mm. persisted over a period of from 4 to 8 min.;—obviously a different mechanism from that which produced a very much greater but purely transitory fall in vapour pressure.

(*b*) Were the alveolar air being cooled by the respiratory passages one would expect this effect to be greater when breathing cold air than hot. It follows that a greater rise in the vapour pressure of the alveolar air is to be expected from a period of apnœa after breathing cold air than after breathing hot air. Within the limits of experimental error this was not found to be the case, the rise with cold air amounting to an average of 0·5 mm. and with hot air to an average of 0·45 mm., in twenty observations.

(*c*) With the exception of the group of observations on the normal vapour pressure of the alveolar air, all the experiments described were performed in an environmental temperature above that of the body. The bronchi were covered with mucous of high viscosity, and it would indeed be surprising if under these circumstances they could be perceptibly cooled by any form of respiratory gymnastics.

Discussion.

This is the first attempt, as far as we know, which has been made to measure directly the vapour pressure of the alveolar air. Mainly in the course of studies on water balance several attempts have been made to measure the total amount of water excreted by the lungs over a given length of time. In all some gravimetric method has been used in none of which is the possibility of condensation of moisture outside the weighing system completely excluded. From this point of view each method has its own particular possibility of error and we will only discuss in brief the conclusions which have been drawn. All are agreed that the expired air is far from saturated with moisture [Galeotti, 1912 and 1915; Loewy and Gerhartz, 1914; Jacquot and Mayer, 1925, 1926], but that the saturation rises with the temperature of the environment [also Rubner, 1898], and is unaffected by the humidity of the environment [also Levi, 1925, *a*, *b*]. Viale [1926] has shown that deep breathing increases water excretion of the lungs, and Galeotti

and Signorelli [1912] have shown the water excretion is increased at high altitudes. From our own results these conclusions would seem to be perfectly reasonable, but it must be remembered that water excretion is largely a function of the volume of air expired, and does not necessarily bear any relationship to the vapour pressure in the alveoli.

Evidently the vapour pressure of the alveolar air is lower by some 2 mm. than the generally accepted value of 47 mm. of Hg. From the experiments described we are able to analyse in some detail the factors responsible for this discrepancy. (*a*) Holding the breath for 45 sec. raises the vapour pressure of the alveolar air by 0·5 mm. irrespective of the temperature of the environment. The possibility of any significant cooling of the alveolar air while passing through the respiratory dead space has been excluded, so we can assume that 0·5 mm. represents the lack of equilibrium between the blood and the alveolar air. Such a gradient seems very reasonable when we consider that Bock and his associates [1924, 1929–30] have shown that with CO_2 a measurable gradient exists between the plasma and the alveolar CO_2. (*b*) The difference between the osmotic pressure of the blood and that of water could only account for a lowering of the vapour pressure of at the most 0·15 mm. (*c*) Binger and Christie [1927] in a large number of observations have shown that, in the anæsthetized dog at least, the lung temperature is always lower than that of the rectum. We have taken 50 such observations at random and have found that the lung temperature is on an average 0·24° C. lower than the rectal temperature. The temperature was measured by thermocouples accurate to $\pm$ 0·02° C. In the same way we found that the blood passing through the lungs was cooled by an average of 0·08° C. on seven simultaneous measurements of the temperature of the right and left heart blood [Binger and Christie]. We have ample evidence then for the assumption that the temperature of the lung is somewhat lower than that of the rectum. If we accept a difference of 0·24° C. as also applying to the human subject, this would represent a fall of 0·6 mm. in the vapour pressure.

These three factors could account for 70 p.c. of the discrepancy between the vapour pressure of the alveolar air, as measured by us, and that which has been assumed in the past. When we consider that there is a difference of about two atmospheres between the osmotic pressure of the yolk and white of an egg [Straub, 1930; Hill, 1931], it would indeed be remarkable if all the factors influencing even the vapour pressure at the surface of a living cell could be analysed.

Effect of environmental temperature.

Even with extreme changes in the temperature of the environment the vapour pressure of the alveolar air was found to follow closely the temperature of the rectum. The same was found to be true with air retained in the lungs for 45 sec. In both the vapour pressure reaches an equilibrium after some 5 to 10 min., while 15 min. or more is necessary for constant readings of the rectal temperature (Figs. 4 and 5). This early rise in the vapour pressure, unaccompanied by any change in the rectal temperature, at first deceived us into the rather attractive hypothesis that a change in the osmotic pressure of the blood and pulmonary epithelium was being reflected. However, the rise in vapour pressure is far greater than could be produced by any such change, and when we take into consideration the lag of the rectal temperature, the phenomenon can be explained more simply by a rise in the temperature of the lung.

Effect of cold and hot water.

It has been suggested [Galeotti, 1912, 1915; Jacquot and Mayer, 1926] that there is some reflex mechanism in the lungs to increase or decrease water excretion, the stimulus being a sensation of cold or warmth. We have been unable to find any evidence for such a reflex. The changes in vapour pressure which we observed with changes in the environmental temperature have already been discussed, and the changes after imbibition of cold or hot water have been shown to be due probably to changes in the temperature of the respiratory dead space.

Effect of hyperventilation.

The lowering of the alveolar vapour pressure which was found after hyperventilation might either be due to cooling of the lungs or to an increased gradient between the alveolar air and the blood. The possibility of cooling the respiratory air passages has already been disposed of, and it seems inconceivable that cooling of the lung could account for the change observed. During the 10–15 sec. period of hyperventilation certainly not more than 0·4 g. of water is excreted, which represents a maximum heat loss of 230 calories, a quantity quite insufficient to lower the temperature of the lung and blood circulating through it by the 3° C. necessary for such a fall in vapour pressure. The lung probably is cooled to a slight extent, but the factor mainly responsible for the fall in vapour pressure must be lack of equilibrium between the alveolar air and the blood, the gradient probably being one of temperature as

well as saturation. Whatever factors may be responsible, the fact remains that the alveolar vapour pressure may be lowered by as much as 9 mm. of Hg by hyperventilation, even in an atmosphere which is warmer than that of the lungs.

This observation would seem to have a direct bearing on any accurate analysis of hæmo-respiratory exchange during work or at high altitudes. With a fall of the alveolar vapour pressure to 39 mm. an error of 0·5 mm. in the pressure of CO_2 and of 1·1 mm. in the pressure of oxygen would result from the use of the standard value of 47 mm. At an altitude of 15,000 ft., with an alveolar oxygen tension of 50 mm. and CO_2 tension of 30 mm. [*Report of the Peru High-Altitude Committee*, 1923], this would result in an error of 0·5 p.c. in the calculated saturation of the arterial blood and an error of 0·01 in the *p*H calculated from the Henderson-Hasselbach equation.

Rapid and shallow breathing.

With a respiratory rate of 60 a minute and depth of 200 c.c. the pressure of water vapour at the end of such a breath was found to average 38·3 mm. Hg, the pressure in the alveoli remaining unchanged. Under similar conditions the tension of CO_2 was found to be 23·2, 22·4 and 20·1 mm. on three occasions, with a CO_2 tension of the expired air of 8·9, 10·0 and 7·8 mm. respectively. The alveolar pCO_2 of this subject averaged 41 mm. on eight observations.

It follows that rapid and shallow breathing is a very efficient mechanism for the elimination of heat by the evaporation of water with CO_2 loss reduced to a minimum. Were the loss of CO_2 to parallel the loss of H_2O a gaseous alkalosis would soon result. In cases of lobar pneumonia with rapid and shallow breathing this does not occur and a dog does not develop tetany when he pants. Presumably this lack of parallelism is to be explained on the score of evaporation in that region where the dead space ends and the alveolar air commences. The evidence we have presented is certainly against any significant lowering of temperature in the respiratory tract and, *a priori*, it would seem that heat loss must be from the pulmonary vascular bed, the whole structure of which, with its amazingly efficient "water-cooled" system, would lend itself to the evaporation of moisture without any significant fall in temperature. When we consider the lack of agreement which exists between what might be called the Haldane school on the one hand, and the Krogh school on the other, as to the boundaries and even significance of the respiratory dead space, it is easy to conceive an area between the

anatomical dead space and the alveoli, hyperventilation of which would produce a loss of water out of proportion to the loss of CO_2. Indeed, if we accept the evidence of Krogh and his associates against homogeneity throughout the alveolar air, such a possibility seems likely, since more H_2O vapour than CO_2 can be given off by a cubic centimetre of blood when hyperventilated. Moreover, in this area heat loss would be from the pulmonary blood itself and not from the bronchial capillaries. Such a mechanism would fully explain the absence of gaseous alkalosis in such conditions as lobar pneumonia, the panting of dogs and any form of thermal polypnœa. It would also explain the subnormal temperature usually found in cases of emphysema with chronic rapid and shallow breathing, with impairment of the function of CO_2 excretion. In repeated observations on five such cases we have found an average temperature of 36·4° C.

Summary and conclusions.

1. A method for the accurate measurement of the vapour pressure of the alveolar air is described.

2. The vapour pressure of the alveolar air is some 2 mm. Hg lower than the value generally accepted. An explanation for this discrepancy is given.

3. The vapour pressure of the alveolar air can be lowered by hyperventilation by as much as 7 mm. Hg. The significance of these observations with regard to hæmorespiratory exchange during conditions of work and at high altitudes is discussed.

4. Changes in the alveolar vapour pressure with the temperature of the environment can be fully accounted for by changes in the systemic temperature.

5. The efficiency and specificity of rapid and shallow breathing as a mechanism for the elimination of heat and conservation of carbon dioxide is described. The application of this mechanism to thermal polypnœa, lobar pneumonia and emphysema is discussed.

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STUDIES IN MUSCULAR ACTIVITY.

VII. Factors limiting the capacity for work.

By D. B. DILL, H. T. EDWARDS and J. H. TALBOTT.

(*From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston.*)

In a dog the energy reserve consists chiefly of carbohydrate and fat. For their utilization oxygen must be supplied and carbon dioxide eliminated. The maximum over-all efficiency is probably from 20 to 30 p.c., most of the energy appearing as heat. Hence, if a constant body temperature is to be maintained, provision must be made for heat dissipation. The experiments to be described indicate that, by suitably varying the conditions, inadequacy in any one of the three factors, fuel supply, oxygen supply or heat dissipation, may limit the capacity for work. In our experiments these three taken singly, or in combinations, are of primary importance, although factors of secondary importance no doubt exist.

Two dogs were trained to run on a motor-driven treadmill. Most of the experiments were carried out on Joe, an immature male of the fox-terrier type weighing 13 kg. Additional experiments were carried out on another dog, a mature female of the Irish terrier type and of the same weight. With the exception of a few early experiments the grade[1] was 17·6 p.c. The rates and other experimental conditions will be given in detail below. Observations were made of: (*a*) heart rate, using a cardiotachometer previously described; (*b*) rectal temperature either with a thermocouple during exercise or with a clinical thermometer after it; (*c*) room temperature; (*d*) blood lactic acid by the method of Friedemann, Cotonio and Schaffer [1927]; (*e*) blood sugar by the method of Folin and Malmros [1929]; and (*f*) morphological properties[2] of the blood. Notes were made of the dog's behaviour, particularly during the onset of exhaustion. The nomenclature of Campos, Cannon, Lundin

[1] According to Tracy [1908] a grade of 1 p.c. is defined as "a rise or fall of 1 ft. measured *vertically* for each 100 ft. measured *horizontally*." This usage, generally accepted by engineers, has been adopted in our calculations although it is not always followed by physiologists, *e.g.* by Smith [1922].

[2] These will be described elsewhere.

and Walker [1929] will be followed in describing the degree of fatigue. The relative humidity was low in all cases, and with a few exceptions (all at low temperature) there was virtually no air movement.

The blood samples were all obtained from the saphenous vein as soon as possible after work stopped. Usually not more than a minute elapsed before the sample was withdrawn. Christensen [1931] suggests that such samples should be called recovery rather than work samples. He shows that the blood-sugar level in man may increase several mg./100 c.c. during the first few minutes of recovery. Three experiments may be cited which show that under the conditions of our experiments only a small change occurs during the first minute of recovery, and hence our values for blood sugar differ but little from work samples. In the first case, a cardiac puncture was made while the animal was running. As soon as blood was obtained, the dog was lifted from the treadmill and with the needle remaining in position a series of samples was collected. In each of two experiments on Joe, one in hypoglycæmia and another in a normal state, a series of samples was obtained during recovery. These results, assembled in Table I, show that changes in blood sugar during the first minute of recovery are unimportant from our point of view.

TABLE I. Change in blood-sugar concentration during recovery. Zero time corresponds to cessation of work.

Experiment 1		Experiment 2		Experiment 3	
Time (sec.)	Blood sugar mg./100 c.c.	Time (sec.)	Blood sugar mg./100 c.c.	Time (sec.)	Blood sugar mg./100 c.c.
−10	86	15	46	10	106
15	92	40	52	30	105
60	97	100	53	90	110
120	92	120	52	130	121
240	92	270	49	160	119
—	—	330	47	—	—
—	—	360	55	—	—

The experiments illustrated in Fig. 1 include several in which either the supply of oxygen or the dissipation of heat determined the work output. In one of these experiments the rate of oxygen supply certainly was the chief limiting factor. In this case, with a room temperature of 16° C., the energy output during work was 352 kg.m. per minute[1] for 7½ minutes, a total of 2640 kg.m. At the end of work there was evidence of a large

[1] The energy output has been calculated from the data of Slowstoff [1903]. He found that the energy requirement for running on an inclined treadmill may be resolved into horizontal and vertical components. For dogs of 12–14 kg. weight the horizontal component is 0·64 kg.m. per metre, and the vertical component 2·92 kg.m. per metre. These are expressed in kilogram-metres per kilogram of body weight.

oxygen debt. The blood lactic acid concentration was high, 74 mg./100 c.c. The laboured and deep character of the respiration during recovery indicated that a high rate of oxygen intake was being maintained. There was confirmatory evidence of an indirect nature. Thus, the rectal temperature

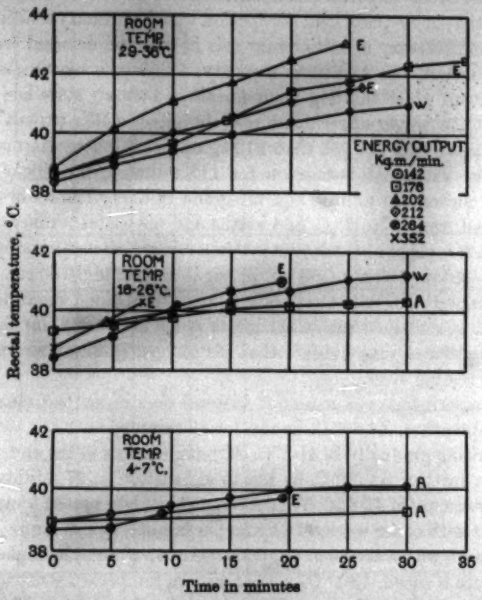

Fig. 1. Rectal temperature in relation to duration of work, intensity of work and room temperature. Exercise was continuous, temperatures being observed with a rectal thermocouple. The degree of fatigue is indicated by the notation of Campos, Cannon, Lundin and Walker [1929]. *A* = Active; ready to continue running. *W* = Weary; panting, but easily able to continue running. *E* = Exhausted; panting most heavily with head down, drops to platform and refuses to rise.

was raised only 1·5° C., an increase which is small in comparison with changes in other experiments shown in the same figure. Furthermore, the blood sugar remained at a high level, 119 mg./100 c.c. Failure was not because of high temperature or lack of fuel. In view of the direct evidence and since no other cause for exhaustion is known, it seems probable that this dog cannot transport enough oxygen to remain in a steady state at

this rate of work. Energy reserves were utilized anaerobically, resulting in accumulation of unoxidized end products. It may be noted in passing that lactic acid accumulation alone is inconclusive evidence of oxygen deficiency in the dog. This subject will be referred to below.

In some of the experiments illustrated in Fig. 1, conditions were adjusted so that inadequate heat dissipation was the primary limiting factor. One of the necessary conditions for this is that the demand for oxygen shall not exceed the transport capacity. While the maximum rate of energy output which this dog can maintain in a steady state has not been determined, a single experiment may be cited which proves that his working capacity is not less than 212 kg.m. per minute. He maintained this output without interruption for 112 minutes, the linear velocity being 187 metres per minute and the grade 17·6 p.c. The room temperature ranged from 4° to 7° C. and at the end his rectal temperature was 39·3° C. A sample of blood drawn within a minute after work stopped was found to contain 105 mg./100 c.c. of sugar and 12 mg./100 c.c. of lactic acid. Apparently, in respect of body temperature, blood sugar and blood lactic acid he remained within the limits which are normal for the resting state, notwithstanding the fact that his oxygen intake was perhaps 15 times the resting level.

This experiment was repeated with all conditions the same except room temperature. At 20° C. he ran for 29 minutes with the rectal temperature rising gradually to 41·3° C. Although weary at the end, he could have gone farther. At 33° C. he ran to exhaustion in 27 minutes with a final temperature of 41·6° C. With a somewhat higher room temperature, 35° C., but with easier work, 176 kg.m. per minute, he ran for 34½ minutes to exhaustion with a terminal temperature of 42·5° C.[1] The highest rectal temperature reached, 43·1° C., was at a room temperature of 29° C. with an energy output of 202 kg.m. per minute for 24 minutes. This was an early experiment with a rate of 233 metres per minute and a grade of 8 p.c. These results prove that work which is easy at low temperatures is difficult at moderate temperatures. As the external temperature is increased, the length of time a given rate of energy output can be maintained diminishes.

There are many indications that exhaustion resulting from work at high temperature is a complex phenomenon. One notes the increase in

[1] In an experiment to be described below (Protocol I), this rate of energy output was maintained for 17 hours with the room temperature near 15° C. and with fuel and water supplied during half-hourly rest periods. A difference of room temperature of 20° C. determines the difference between a steadily increasing body temperature ending in exhaustion and a constant slightly elevated temperature with virtually tireless performance.

effective area from which heat is dissipated and simultaneous increases in salivary flow, respiratory rate and respiratory volume. These changes call for a greater oxygen supply both for the muscles involved in breathing and for the heart. With a rate of oxygen supply already near the

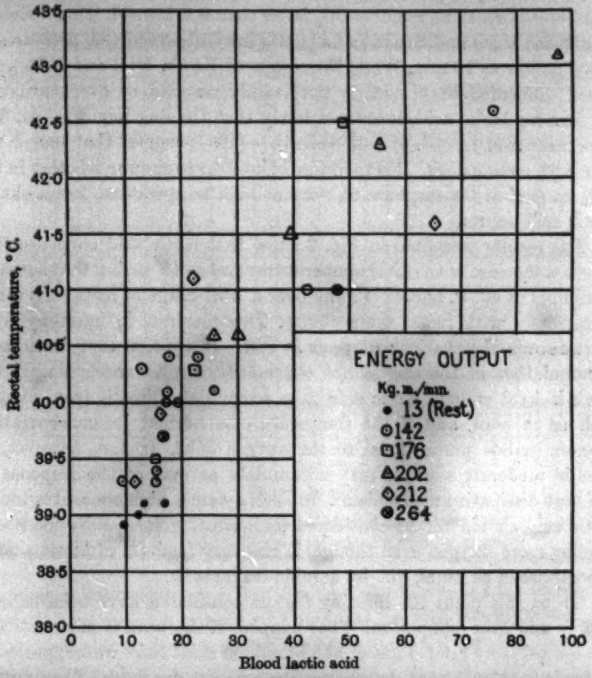

Fig. 2. Blood lactic acid in relation to rectal temperature. The blood sample was drawn at the end of a work period of at least 15 minutes' duration and rectal temperature was observed simultaneously.

upper limit, this further demand may result in breakdown. Under such conditions, before the rectal temperature has reached a high value, exhaustion may come as a consequence of anaerobic production of mechanical energy for dissipating heat. At present the evidence for this statement must remain indirect since, as intimated above, lactic acid

concentration is not an infallible measure of oxygen debt in the case of the dog.

The highest rectal temperatures have been reached with rates of oxygen intake well below the maximum. In such cases, as body temperature rises, polypnœa and related changes become more pronounced. This forced breathing removes CO_2 faster than it is formed. Under similar conditions Rice and Steinhaus [1931] have observed CO_2 pressures in blood as low as 10 mm. While the results of Bock, Dill and Edwards [1932] indicated that men do not readily respond to overventilation by forming lactic acid, it seemed likely that the dog might do so. The experiments of Knudson and Schaible [1931] suggest that such is the case with resting dogs. It is therefore of interest to enquire whether in the dog, as part of the response to work at high temperature, formation of lactic acid occurs.

The results presented in Fig. 2 show that lactic acid concentration does not increase until rectal temperature exceeds 40° C., but that between the limits of 40° C. and 43° C. and over a wide range of metabolic rates, it increases with rectal temperature. This result is in harmony with Knudson's observations on dogs at rest. It appears that lactic acid accumulation in the dog is not necessarily due to anoxemia. If the work is hard enough lactic acid may accumulate directly from oxygen lack as in man, and if the temperature is normal its concentration is more or less proportional to the oxygen debt. In rest, however, or even in moderate work, it may accumulate as part of the response of the heat dissipative mechanism[1]. In such a case a high concentration of lactic acid should not be considered as indicating incapacity for transporting more oxygen even though in recovery removal of lactic acid is accomplished as usual, viz. by aerobic resynthesis.

Up to this point the limiting factors considered have been oxygen supply and heat dissipation. What happens if the work is moderate and the temperature low? First of all the animal must have water; provided water is supplied, what determines the capacity for work? The limiting

[1] The results of Rice and Steinhaus [1931] indicate a reduction in alkali reserve during work accompanied by increase in rectal temperature. It is possible with certain assumptions to calculate the decrease in alkali reserve (the total CO_2 of oxygenated blood when $pCO_2 = 40$ mm.) from their data. Thus in their Fig. 1 the pCO_2 was reduced from 40 to 10·5 and the ($BHCO_3$) from 24 to 6·7 mM/litre. Assume the same oxygen content and an oxygen capacity of 20 volumes per cent. in these blood samples. On the basis of these assumptions and the known properties of dog blood [see Dill, Edwards, Florkin and Campbell, 1932] the reduction in alkali reserve was about 7 mM. Since the pH was nearly constant such a change in alkali reserve implies an increase in blood lactic acid of about 60 mg./100 c.c.

factor seems to be merely the quantity of easily available fuel; the principal evidence for this is found in Fig. 3. These experiments were carried out according to a uniform plan. The run began either after a 36 hours' fast or 1 hour after a heavy carbohydrate meal. In the first case, exercise was carried on to exhaustion; in the second case, fuel was

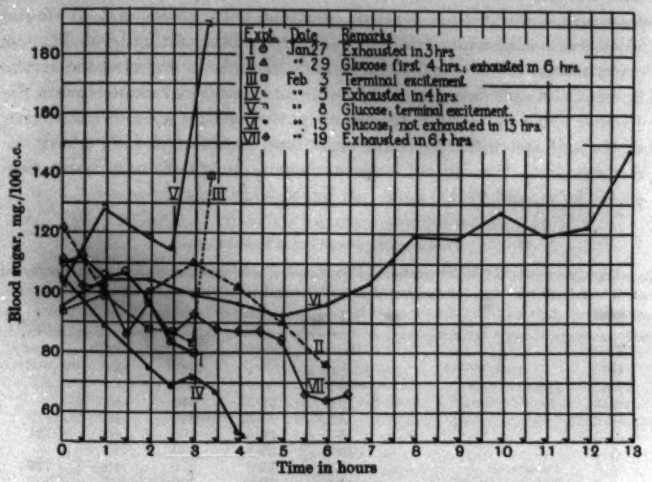
Expt. Date Remarks
I ○ Jan 27 Exhausted in 3 hrs.
II △ " 29 Glucose first. 4 hrs., exhausted in 6 hrs.
III □ Feb 3 Terminal excitement.
IV ◇ " 5 Exhausted in 4 hrs.
V ⌐ " 8 Glucose, terminal excitement.
VI • " 15 Glucose, not exhausted in 13 hrs.
VII ◆ " 19 Exhausted in 6+ hrs.
Blood sugar, mg./100 c.c.
180
160
140
120
100
80
60
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time in hours

Fig. 3. Performance in relation to blood sugar. Observations were made at 30-minute intervals during rest periods of 5 minutes' duration.

supplied at intervals[1]. In either case the schedule called for 25-minute runs with intervening periods of 5 minutes for withdrawing blood, observing rectal temperature, supplying water and, in some cases, fuel. The

[1] The fuel used (except in one case when sterile glucose was injected and in a second case when glucose was given by stomach tube) was a candy referred to as glucose candy. Analysis was made after the experiments were concluded and it was found to contain 16 p.c. glucose and 72 p.c. sucrose + polysaccharide. According to the manufacturer it also contained citric acid and artificial colour and flavour. It contained by analysis 0·17 p.c. ash and less than 1 mg. of combined phosphorus per 100 g. The dog is able to utilize this fuel alone during long periods of exercise, indicating that added phosphate is unnecessary, contrary to the suggestion of Dische and Goldhammer [1932]. Furthermore, in order to utilize sucrose the secretion of digestive juices is necessary. For this purpose it is particularly important to maintain a low temperature. Nausea and vomiting are likely consequences of running a dog with a full stomach, if the temperature is high.

dog ran 50 minutes of each hour, putting out in each of these experiments 142 kg.m. of energy per minute while running.

Under these conditions the best performance recorded in Fig. 3 when working without fuel was a run of 6½ hours to complete exhaustion. Four days before this, when fuel was supplied, the same dog ran for 13 hours and was not exhausted at the end. The room temperature was 15° C. in each case, the rectal temperature remained between 39° and 40° C. and blood lactic acid concentration remained near the resting level. There is every reason to believe that exhaustion came from depleted fuel in the 6½-hour run and that the administration of easily available fuel in the 13-hour run resulted in twice the output of energy without exhaustion at the end and with the blood-sugar level maintained at or above the resting level. The actual energy outputs in these two cases were 46,150 kg.m. and 92,300 kg.m. per kg. of body weight.

PROTOCOL I. Record of a 17-hour run.

Energy output, 176 kg.m. per minute or a total of 150,000 kg.m.; a 5-minute rest period each half-hour was used for making observations and supplying water and fuel.

Time (hr.)	Remarks	Temperature		Intake		Blood sugar mg./100 c.c.
		Room (°C.)	Rectal (°C.)	Water (c.c.)	Fuel (g.)	
0	Start	15·5	38·7	—	—	—
0–1	—	15·0	39·5	340	40	—
1–2	—	14·7	39·5	250	40	112
2–3	—	15·5	39·6	180	40	131
3–4	—	15·8	39·7	330	40	—
4–5	Defæcated	16·0	39·6	270	40	127
5–6	Refused candy	15·6	39·9	270	0	132
6–7	Urinated	14·8	39·8	120	40	—
7–8	Defæcated	14·8	39·8	30	40	120
8–9	—	14·0	39·7	180	20	111
9–10	—	12·9	39·6	50	20	—
10–11	—	15·7	40·0	105	20	107
11–12	Refused candy	14·4	39·6	215	0	—
12–13	Refused candy	13·6	40·0	100	0	103
13–14	Glucose by stomach tube	13·6	39·8	295	42	—
14–15	Glucose by stomach tube	13·4	39·7	355	50	94
15–16	Refused candy	14·0	39·7	100	0	—
16–17	Tired but not exhausted	13·7	39·5	100	0	101

Three later experiments, not illustrated in Fig. 3, may be referred to briefly. With the treadmill running at the same rate, our second dog, with fuel supplied, ran for 9 hours. By that time she was becoming tired and her temperature had risen to 41·3° C. At this time the rate was decreased to 92 metres per minute, with the result that rectal temperature soon returned to normal and she was able to run easily for 15 more hours. At the end of 24 hours she was not exhausted notwithstanding an energy output

of 64,000 kg.m. during the first 9 hours and 79,000 kg.m. during the succeeding 15 hours, a total of 143,000 kg.m.

In the next experiment, detailed in Protocol I, Joe was used as a subject with the rate of work output one-fourth greater than in the experiments of Fig. 3. This higher rate of work output, 176 kg.m. per minute, is approximately the same as that which Rice and Steinhaus [1931] used. Under their experimental conditions, "rarely can a dog run continuously for 30 minutes." Joe had already maintained a still higher work output for 112 consecutive minutes (as already stated), and in this case he ran for 17 hours with fuel supplied and was not exhausted at the end. The total work output was 150,000 kg.m.

In the final experiment conditions were the same except that no fuel was supplied until after 4¼ hours' work. As may be seen in Protocol II,

PROTOCOL II. Record of a run demonstrating the effects of withholding and supplying fuel.

Conditions as in Protocol I except that food had been withheld for 36 hours and none was supplied until the onset of exhaustion. Then (during a rest period of 8 minutes instead of 5) 40 g. of candy restored the animal to an active state and simultaneously brought the blood sugar to its normal level. The experiment was discontinued when it appeared that the dog had returned to a steady state.

Time (hr.)	Remarks	Temperature		Intake		Blood sugar mg./100c.c.
		Room (°C.)	Rectal (°C.)	Water (c.c.)	Fuel (g.)	
0	Start	13·5	38·5	—	—	78
0–1	—	14·8	39·8	120	0	88
1–2	—	16·6	40·0	120	0	82
2–3	—	16·0	39·7	140	0	71
3–4	Fan started	17·0	39·5	155	0	50
4–4¼	Exhausted	16·0	39·6	10	0	46
4¼–4½	Rest period; candy supplied	—	—	—	40	—
4½–5½	Ran easily	15·8	39·8	420	20	127
5½–5¾	Ran easily	14·3	39·8	100	0	106

he was unable to run longer without fuel. He was revived by 40 g. of candy and was able to resume work within 8 minutes. The experiment was discontinued 1½ hours later, for the dog seemed to be in good condition and able to continue for hours with fuel supplied.

A study of the reasons for exhaustion in these experiments naturally leads to a consideration of the heart rate. The importance of the cardiotachometer for this purpose is revealed by Fig. 4. The rapid decrease in heart rate during recovery shows that counts made in the usual way at the end of work may be 100 beats per minute too low. One rarely reads of a heart rate greater than 150 or 160 in the dog after work stops.

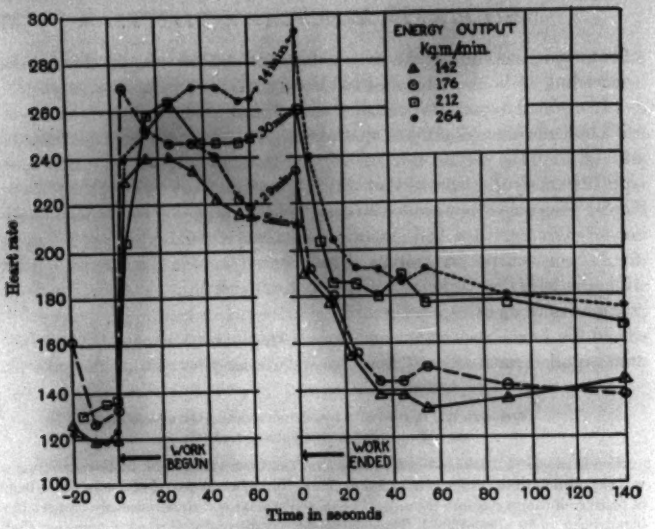

Fig. 4. Heart rate during work and recovery.

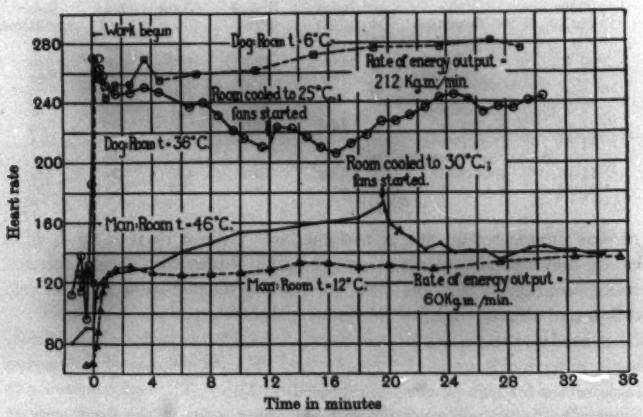

Fig. 5. The relation of heart rate to external temperature. With the onset of exhaustion during work in a hot room the heart rate of the dog decreases, that of man increases. As work continues after the room is cooled, the heart rate of the dog increases, that of man decreases.

Actually the rate after a steady state is reached is closely proportional to the rate of energy output and may be as high as 300 beats per minute.

It is not proposed at this time to present detailed observations on the heart rate during all these experiments. Two other sets of records will be presented briefly. The first of these, given in Fig. 5, shows the difference in response of man and dog to heat. As man approaches exhaustion at a high external temperature his heart rate rises and commonly the point of exhaustion coincides with the attainment of his maximum heart rate.

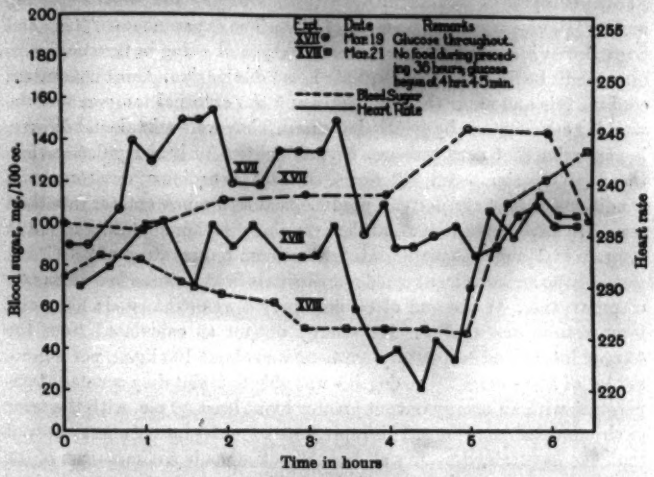

Fig. 6. Heart rate and blood sugar. Rest periods were interposed as described in the legend of Fig. 3.

In this case, fans were turned on the man and the room was cooled suddenly. Work was continued at the same rate, the heart rate decreased 30 to 40 beats per minute and within 3 minutes the subject had passed from a state of exhaustion to one of comfort. The dog, on the other hand, showed a decrease in rate with the onset of exhaustion, and after the room was cooled recovery (which was slower than in man) was accompanied by an increase in heart rate. Similar observations have been made on other occasions; evidently the control of the heart rate under these conditions is different in the two species.

Fig. 6 shows simultaneous observations of heart rate and blood sugar in two experiments. While there are large changes in heart rate which are

not parallel to the observed changes in blood sugar concentration, it is nevertheless a fact that in the experiment in which no fuel was given until the onset of exhaustion the minimum heart rate was reached shortly before candy was given. As the blood sugar increased from 50 to 100 mg./100 c.c., the heart rate increased from 220 to 235 beats per minute. Further experiments on this subject are planned.

The experimental results are of such a character that little discussion is necessary. The literature has been reviewed recently by Rice and Steinhaus [1931], by Campos, Cannon, Lundin and Walker [1929], and by Dische and Goldhammer [1932]. The experiments of Rice and Steinhaus indicated that the energy output of a dog in treadmill running might be limited by inadequate heart dissipation. Our experiments confirm this and show that by decreasing the external temperature the working capacity can be greatly increased. They were mistaken, however, in supposing that man possesses better facilities for heat regulation than the dog. The significant difference, at least in ordinary environmental conditions, is that the dog can produce heat at a much greater rate than man. We have found that an athlete running on a horizontal treadmill at the rate of 233 metres per minute with a room temperature of 22° C. and virtually no air movement could not dissipate heat quite as fast as its rate of production. At the end of 80 minutes he was exhausted; his rectal temperature was 40·6° C. His energy output as calculated from the oxygen intake and respiratory quotient was about 100 kg.m. per minute per kg. of body weight. The dog Joe was able to maintain a constant temperature with an energy output greater by at least 50 p.c. with the same environmental conditions. This represents a greater rate of energy output than can be attained by any athlete. We have made a comparison of the working capacity for short periods of this dog with that of a trained runner. Joe ran at the rate of 311 metres per minute on a grade of 17·6 p.c. for 7½ minutes to exhaustion. A mile runner, able at the time to run a mile in 4 minutes and 30 seconds, was exhausted with the treadmill at the same grade after 6 minutes at the rate of 125 metres per minute. Evidently this dog has a capacity for expending energy which is 2½ times greater than that of an athlete. It is not surprising, therefore, that heat dissipation is a more common limiting factor than with man[1].

The experiments of Eagle and Britton [1932] showed that the

[1] Dogs no doubt differ greatly in heat dissipating capacity. Perhaps Joe has a greater than average ability to dissipate heat on account of his small size and short hair. The dogs used by Rice and Steinhaus were twice as large and possibly were handicapped on this account.

administration of their cortico-adrenal extract to normal dogs increased their capacity for work. We are indebted to Dr Eagle for supplementary information regarding their experimental conditions. No water was given to their dogs during exercise and the room temperature was about 25° C. They report some decrease in blood sugar, although this was not simply related to degree of exhaustion. The total energy output before cortico-adrenal extract was about 15,000 kg.m. and from 20,000 to 25,000 after. The work output was at a lower rate, but even with cortico-adrenal extract the total output was only one-sixth as great as in our 17-hour experiment on Joe. The rate was the same and the total work output about two-fifths as great as in the 9-hour experiment on our second dog. The most reasonable interpretation of the effect on working capacity of cortico-adrenal extract is that it is indirect, possibly helping in mobilizing fuel, possibly eliminating unpleasant sensations arising from thirst, hunger and overheating. The same may be said of the similar effect of adrenaline on working capacity. In the most successful experiment of Campos, Cannon, Lundin and Walker [1929] in demonstrating the effect of adrenaline, the rate of work output was four-fifths as great and the total work output one-third as great as in our 17-hour experiment. Neither extraneous adrenaline nor cortico-adrenal extract *per se* is necessary even when the exercise is of long duration.

Summary.

A study has been made of three factors which may limit the capacity of a dog for work—external temperature, supply of oxygen, and supply of fuel. A fourth factor perhaps of equal importance is the supply of water. The water intake has been observed in all these experiments, but the limiting effect of inadequate water intake has not been studied.

With external temperature low and oxygen supply adequate and when fuel and water are supplied, the performance of a dog is virtually tireless. In one case a dog ran for 17 hours with 5-minute rest periods each half-hour. In the 17 hours he ran 132 km. (82 miles) and climbed 23 km. (14 miles) with a total energy output of 150,000 kg.m. per kg. of body weight. His working capacity was increased more than three times by supplying fuel. His energy output in this run was three times greater than in any recorded experiment showing the influence of adrenaline injections on working capacity, and six times greater than in similar experiments with cortico-adrenal extract. Evidently the dog does not require an extraneous supply of either of these hormones for work of many hours' duration.

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PHYSIOLOGICAL LEUCOCYTOSIS.

II. Post-prandial leucocytosis and Widal's hæmoclasic test for hepatic efficiency.

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Various opinions have been expressed on post-prandial leucocytosis, and Widal's hæmoclasic test for hepatic efficiency.

Among recent authors, Beaumont and Dodds [1931] reported that "Widal's hæmoclasic reaction...is held to be quite reliable," while Rolleston and McNee [1929] deprecated the test and quoted Shaw's views that "the test lacks any theoretical basis, and is of no value in the diagnosis of hepatic efficiency." Further work was clearly necessary.

Before Shaw's investigation [1925] most workers confirmed Widal's work, although its clinical significance was uncertain. The existence of post-prandial leucocytosis is denied by Shaw, who suggested in a later paper [1927] that the observed leucocytosis was due to the normal diurnal variation of the leucocytes. While Shaw's work would appear to be based upon careful experimental work, it has not been repeated. Shaw's theory of the diurnal variation is not new. It had been advanced 27 years previously by Japha [1900], who however concluded that there was a post-prandial leucocytosis quite distinct from the diurnal variation.

Method.

In the present work the technique described in Part I [Martin, 1932] was used.

In order to make sure that the results obtained were due to the ingestion of protein and not to the normal variation in the leucocyte count, a series of control experiments was carried out on fasting and resting patients (Table I). Blood samples were taken at approximately the same intervals as in the subsequent experiments.

TABLE I. Control experiments. Variations in the leucocyte count of fasting subjects.

Time in min.	Leucocytes per c.mm.					
	Case 1	*Case* 2	*Case* 3	*Case* 4	*Case* 5	*Case* 6
0	8,400	9,000	7,400	6,600	6,400	7,700
25	8,200	9,300	6,700	7,800	6,200	7,300
50	8,600	8,800	6,700	6,800	6,800	7,200
100	8,600	9,200	6,200	7,800	6,500	7,200

I. THE EFFECTS OF A FULL MEAL ON THE LEUCOCYTE COUNT.

The effects of a full meal on the leucocyte count were investigated on six normal subjects and three cases of hepatic disorder. Of the latter, case 13 was one of mitral disease with enlarged liver and jaundice, and Nos. 14 and 15 were cases of multilobular hepatic cirrhosis with ascites. The patients were confined to bed and starved from the previous midnight. A meal of meat, potatoes, cabbage, milk pudding and half a pint of milk was given at 12.15 p.m. A sample of blood was taken immediately before the meal. Three more samples were taken at 20, 50 and approximately 110 min. after the meal. The results obtained are given in Table II.

TABLE II.

Normal subjects				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
Case 7		*Case* 10		*Case* 13	
	8,000		10,400		13,400
20	9,000	20	10,700	20	14,200
50	8,800	50	9,700	50	12,200
120	8,300	100	8,700	120	11,500
Case 8		*Case* 11		*Case* 14	
	8,400		6,200		6,000
20	9,300	20	6,300	20	5,200
50	9,200	50	6,200	50	6,800
110	9,500	100	6,400	100	7,500
Case 9		*Case* 12		*Case* 15	
	7,400		7,900		5,500
20	7,000	20	6,500	20	4,200
50	6,200	50	7,300	50	4,100
100	5,700	100	6,200	100	5,300

The leucocyte counts taken 20 min. after the meal yield a rise in four out of the six normal cases, and a fall in two of the three hepatic cases. The later counts are quite irregular. A comparison of Tables I and II shows that the leucocyte variations after the meal are similar in degree and sense to those obtained from the fasting subjects.

II. The effects of the ingestion of milk on the leucocytes of the blood.

Widal and co-workers [1920] claimed that the ingestion of 8 oz. of milk would cause a leucocytosis in healthy persons and a leucopenia in patients with hepatic disorder. Both Wilson [1922] and Feinblatt [1923] confirmed this on a large number of patients.

This test was carried out on six normal subjects (Nos. 16–21), one case of syphilitic cirrhosis of the liver with ascites (No. 22), one of carcinoma of the gall bladder with hepatic metastases and jaundice (No. 23), and one of multilobular cirrhosis with ascites (No. 24). The patients were starved overnight and confined to bed on the day of the test. Ten ounces of milk were given about 10.0 a.m. Samples of blood were taken just before giving the milk and at approximately 25, 50 and 80 min. afterwards.

Table III.

Normal cases				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
Case 16		*Case* 19		*Case* 22	
	7,000		6,100		12,500
25	7,300	25	6,800	25	12,300
50	7,500	50	6,300	65	10,700
85	6,000	75	6,800	100	12,500
Case 17		*Case* 20		*Case* 23	
	7,400		7,800		7,800
25	7,600	25	10,000	25	7,600
75	6,300	50	8,200	85	7,700
120	7,600	80	9,000	120	9,300
Case 18		*Case* 21		*Case* 24	
	7,700		6,400		9,600
25	7,900	25	6,900	25	10,100
50	6,100	50	8,000	50	10,800
80	6,200	90	8,900	80	10,900

In all the normal subjects there is an increase of leucocytes during the first 25 min., and in the hepatic cases there is a corresponding diminution in two out of the three cases. The 50 and 80 min. readings are quite irregular and it is not possible to draw any conclusions from them. In all the above cases the variations lie within the limits of the normal resting patient.

III. The effect of the ingestion of peptone on the leucocyte content of the blood.

Most of the investigators of post-prandial leucocytosis, including Widal and co-workers [1920] and Schiff and Stranski [1921], attributed the phenomena to protein degradation products. The results just tabulated fell within the range of the fasting patient. If products of protein digestion could be given in large amounts, it was hoped that the post-prandial leucocytosis could be so increased that the counts obtained would lie outside the normal range. Peptone was therefore used, since it is non-toxic and can be given in large quantities. Six normal persons (Nos. 25–30), one case (No. 31) of syphilitic hepatic cirrhosis with jaundice, one case (No. 32) of jaundice due to malignant nodules in the liver, and one case (No. 33) of advanced multilobular portal cirrhosis with ascites, were examined. They were starved overnight. A blood sample was taken and 1½ oz. of Fairchild's peptone, dissolved in the minimum amount of water (1½ oz.), were administered. Blood counts were made at 30, 60 and 100 min. afterwards (Table IV).

Table IV.

Normal cases				Hepatic cases	
Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.	Min.	Leucocytes per c.mm.
Case 25		*Case* 28		*Case* 31	
	7,900		11,900		3,400
20	9,000	30	12,800	30	3,300
50	8,600	60	11,900	60	3,500
80	8,100	90	12,300	85	3,000
Case 26		*Case* 29		*Case* 32	
	9,000		9,200		12,300
20	10,100	30	7,500	30	12,900
50	9,900	60	9,100	60	14,000
75	9,900	120	11,700	85	12,000
Case 27		*Case* 30		*Case* 33	
	8,500		6,600		6,800
20	9,000	30	7,000	30	7,400
50	9,000	60	7,000	60	7,000
70	10,000	100	8,000	80	4,000

All the normal subjects, except No. 29, show a rise after the ingestion of peptone, which is most marked at the end of the first half-hour. Of the hepatic cases one showed a fall during the first half-hour and two showed a rise. As in the experiments on the effects of a full meal and of milk the variations are of the same degree as in the fasting patient.

SUMMARY.

The effect of giving a full meal, milk and peptone to normal subjects and patients with advanced hepatic disorder has been studied. The results fell within the normal range of the leucocyte count of the fasting subject.

I should like to thank Prof. F. E. Tylecote for his encouragement and help, Dr F. H. Smirk for suggestions and Miss M. J. Rickards, B.Sc., for help with the counts.

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THE ACTION OF ADRENALINE UPON THE CARDIAC VAGUS CENTRES.

By G. STELLA.

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It has been known, since Oliver and Schäfer's experiments [1894], that adrenaline injected intravenously causes a rise of arterial pressure together with slowing, often considerable, of the heart rate. The latter is brought about through vagal inhibition, since it is no longer observed, after cutting both vagi centrally to the heart. As to the direct causes of this excitation of the inhibitory cardiac centres, opinion is still divided: some authors think that it is due to a direct effect of the drug upon the cardio-inhibitory centres, others explain the slowing simply as a reflex response to the rise of arterial pressure. The latter view is held, among recent workers, by C. Heymans, as will be seen below [1929, 1930]. Biedl and Reiner [1898] injected adrenaline into the peripheral end of a carotid artery and noticed an immediate effect upon the heart rate, which was slowed down to a considerable extent: this effect, however, was of very short duration: the rate soon recovered, to slow down again in the usual manner coincidently with the rise in general blood-pressure. They concluded that the second slowing of the heart represented a reflex from arterial hypertension, and that this was the mechanism of bradycardia in the case of intravenous injection. Also Gerhardt [1900] pointed out that adrenaline bradycardia usually appears only after the arterial pressure has risen to a certain degree, and believed the latter to be the only cause of the former. In some experiments, however, in which very small doses of adrenaline had been used he observed a well-marked heart slowing not accompanied by any rise of general blood-pressure.

It seemed difficult, however, to distinguish between a direct and an indirect action of adrenaline in these experiments: even in the case observed by Gerhardt of bradycardia without hypertension, the cause might have been a more abrupt rise of the arterial pressure at each

systole, as a consequence of the adrenaline acting directly upon the heart: whereas, on the other hand, the usual coincidence of bradycardia with hypertension by no means excludes a concurrent central effect.

Anrep and Starling [1925] studied the problem on dogs with the head perfused by means of a heart-lung preparation. They found that adrenaline injected into the separate head circuit and thus incapable of directly affecting the aortic pressure produced a well-pronounced diminution of the heart rate coincident in time with the adrenaline bradycardia, which follows an intravenous injection in the whole animal.

The same results were obtained by Anrep and Segall [1926], using the technique of the innervated heart-lung preparation, in which the systemic blood-pressure was kept under control and constant throughout the action of adrenaline.

The head circuit, in the experiments of Anrep and Starling, and of Anrep and Segall, however, included the carotid sinus with all its nervous connections intact. Now during the action of adrenaline the peripheral vaso-constriction in the head caused a rise of carotid pressure; this, however, was not the only cause of the observed bradycardia as was shown by the fact that the latter was still produced, although to a less extent, when care was taken to prevent the rise of carotid pressure and keep it constant throughout. Moreover, Volhard [1930], who repeated Anrep and Segall's experiments on dogs with denervated carotid sinus, obtained the same results, *i.e.* that adrenaline causes slowing of the heart, independently of increase of pressure in the territories of the depressors and carotid sinus nerves: this view of adrenaline bradycardia independent of peripheral hypertension is opposed by C. Heymans: as a crucial test to this thesis he points out [1930] that if both depressor and carotid sinus nerves be previously cut, adrenaline in the usual doses completely fails to cause any slowing of the heart, in spite of the fact that both vagi are left intact and in good condition.

The reason for the difference between his results and those of Volhard is to be sought, according to Heymans, in the different conditions under which the head was kept in the two cases.

Method and experimental results.

The experiments of Heymans, although they may be accepted as showing that adrenaline does not actually excite the cardio-inhibitory centres, cannot be taken as a proof that it has no effect on these. It is still possible, for instance, that this substance increases the reflex

excitability of the vagus centres, and that the fundamental difference between Heymans's and Volhard's techniques is not the perfusion of the head in the latter's with defibrinated blood, but rather the fact that the afferent nerves for the reflex regulation of the heart rate had been left intact in one case [Volhard] and cut in the other.

In the present work the effect of adrenaline upon the degree of reflex slowing of the heart, elicited by a rise of pressure in the region of the carotid sinus, or in the territory of the depressor nerves was investigated.

(1) Adrenaline and cardiac reflexes from the carotid sinus.

Dogs were used throughout, anæsthetized with morphine (0·5 cg. per kg.) and urethane (0·30 g. per kg.). One or both carotid arteries at the region of the carotid sinus were perfused with defibrinated blood: for this purpose cannulæ were placed one in the common carotid about 2 cm. below the bifurcation, for the inlet of the perfusion fluid, a second in the external carotid, distal to the origin of the lingual artery, for the outlet, and a third in the lingual artery and connected to a mercury manometer—all other arterial branches leaving the carotid between the cannulæ having been previously tied, including all nervous connections, with the exception of the nerve of Hering. For the perfusion, a Dixon pump with a variable resistance in series was employed, as already described [G. Stella, 1931].

When only one sinus was perfused, the other was denervated: also the depressor nerves were carefully cut on both sides, just below the origin of the laryngeal superior [Koch's technique, 1931]. When the last operation was successful it was found that, if adrenaline was injected, while the pressure in the carotid sinus was maintained constant and low (40–50 mm. Hg), the resulting rise in general arterial pressure was not accompanied by any cardiac slowing. That this negative result was not due to an accidental destruction of the efferent cardiac fibres in the vagus trunk was proved by subsequently raising the pressure in the perfused carotid sinus, when the usual effect of slowing of the heart rate was produced. The injection of adrenaline, while the carotid sinus pressure remained low and constant, was thus used at the beginning of each experiment as a routine test for checking the complete destruction of the depressor fibres running with the vagus; the same test was also carried out at the end of the experiment to make sure that no recovery of damaged depressor fibres had meanwhile taken place.

The absence of cardiac slowing from adrenaline, under the conditions

above described, is in agreement with Heymans's [1930] findings, *i.e.* absence of cardiac inhibition from adrenaline after denervation of the carotid sinuses and section of the depressor nerves: in fact when the pressure in the carotid sinus is maintained at 40–50 mm. Hg and not pulsating, the depressor impulses from the sinus may be considered to be practically cut off [Heymans and Bouckaert, 1930; Stella, 1931;

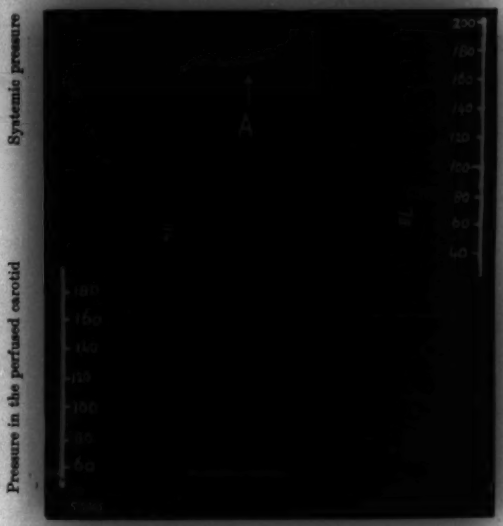

Fig. 1. Dog 11 kg. With both depressors cut and left carotid sinus denervated. Right carotid sinus perfused. Upper tracing: pressure in the femoral artery (mercury manometer). Lower tracing: pressure in the perfused sinus (manometer in the lingual artery). At *A* 1 c.c. of 1 : 40,000 adrenaline was injected into the femoral vein.

Bronk and Stella, 1932]. On the other hand, it was found that if, soon after the intravenous injection of adrenaline, the pressure in the perfused carotid sinus was raised to such a level as would usually cause only a slight slowing of the heart rate, the degree of slowing was definitely greater and sometimes considerably greater.

Fig. 1 shows one such experiment: here only the right carotid sinus was perfused, and the left denervated. It will be seen that a rise of the perfusion pressure from about 60 mm. Hg to 165 mm. before and after

injection of 1 c.c. of 1 : 40,000 of adrenaline (at *A*) has a quantitatively different effect upon the heart rate.

Again, if adrenaline is injected while the pressure in the sinus is high, a further retardation of rate is seen to occur, as shown in Fig. 2.

In other cases the effect was much more pronounced, and this was especially so when both carotid sinuses were perfused: thus in the instance reproduced in Fig. 3 *a* and *b*, the difference in the slowing of

Fig. 2. Dog 10·5 kg. Conditions same as in Fig. 1. At *A* injection of 1·1 c.c. of 1 : 40,000 adrenaline in femoral vein.

the heart before and after adrenaline almost reaches 300 p.c. Fig. 3 *b* is also of interest because it shows the fact already described, *i.e.* that adrenaline under these conditions had no effect upon the heart rate until the pressure in the sinus was raised.

Two obvious possibilities presented themselves as to the causes for the observed results, *i.e.* either an action of adrenaline upon the cardio-inhibitory centres, making them more excitable to afferent stimuli, or else a peripheral action making the heart more responsive to vagal inhibitory impulses, or both. A hint as to a possible peripheral action of adrenaline is given by the work of M. Kuroda and Y. Kuno [1915] who

found, in the dog heart-lung preparation, that after the addition to the blood reservoir of 0·03–0·05 mg. of adrenaline stimulation of the vagus was as effective as, or even more effective than before. Before them X. Mathieu [1904] found that in dogs with both vagi cut, and in which one vagus was continuously stimulated, the injection of 0·5 mg. adrenaline caused a further slowing of the heart. The doses used by the latter

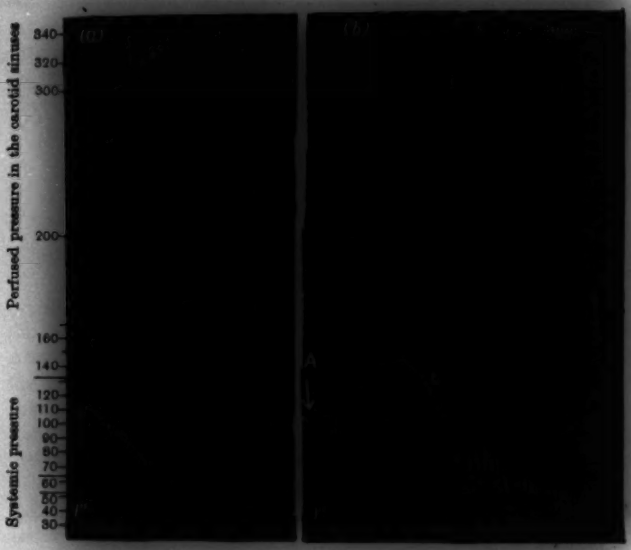

Fig. 3 *a* and *b*. Dog. 13 kg. Both depressors cut. Both carotid sinuses perfused. At *A* intravenous injection of 0·5 c.c. of 1 : 10,000 adrenaline.

author are, however, far above those employed in the present experiments, while the experimental conditions obtained by Kuroda and Kuno are not directly comparable with the present ones; therefore a number of experiments were performed on dogs anæsthetized in the manner already described and with both carotid sinus nerves and vagi cut; one vagus or the other was then stimulated faradically for short times and at regular intervals: it was found that adrenaline injected into the femoral vein in the amounts equal to those employed in the previous experiments did not cause any measurable change in the degree of heart

inhibition from vagus stimulation. It seems, therefore, that the increase of reflex inhibition of the heart described above must be due to a central action of adrenaline, produced either directly or indirectly, perhaps through vascular changes in the brain. This conclusion is further supported by experiments described below in which adrenaline was made to

Fig. 3 *c*. Same as Fig. 3 *a* and *b*. The perfusion pressure is raised again, after 2 min. from *A* (see Fig. 3 *b*). It shows that the effect upon the heart rate is only slightly bigger than before the injection (Fig. 3 *a*).

circulate through the head, but prevented from coming into contact with the heart.

It will be seen in Fig. 2 that the first effect of adrenaline in causing the reflex slowing of the heart rate appears after a certain interval of time after the injection, and coincides with a moment at which the arterial pressure has risen to some extent. This same interval is seen in the intact animal. It appears, therefore, that in the latter bradycardia does not set in only as a result of an appropriate rise in arterial pressure,

but also because at that moment the adrenaline is displaying its central effect.

In the intact animal the degree of adrenaline bradycardia is great at first, and diminishes subsequently: this again, on the basis of the present experiments, must be interpreted mainly as a consequence of the subsidence of the action of adrenaline upon the cardiac centres: in fact, if the pressure in the carotid sinus be raised at different times after the end of the injection of adrenaline it is found that the slowing is of very different degrees, great at first, and then smaller and smaller. Fig. 3 *c*, for instance, shows that the effect of adrenaline is nearly over after 2 min. from the injection.

(2) Reflex slowing of the heart from rise of pressure in the territory of the depressor nerves.

These experiments were performed on the innervated heart-lung preparation of dogs [Anrep and Segall, 1926]. The head was perfused with defibrinated blood by means of a Dixon pump, through the brachiocephalic artery; the blood from the reservoir going partly into the inferior vena cava and heart-lung, partly to the pump and head. After circulating through the head the blood came back to the heart through the superior cava and through the lungs back to the reservoir. Previous to the establishment of the artificial circulation, the carotid sinus on both sides was carefully denervated. The vagi and depressor nerves were instead left intact.

The experimental conditions thus obtained allowed a complete control of pressure and of the rate of circulation in the head and in the arch of the aorta. It was thus possible to vary the pressure in the aorta within very wide limits, while keeping that in the head practically constant. In complete agreement with the results described in the first part of this paper, it was found here, too, that the reflex slowing from hypertension in the left heart and aorta was always more marked while adrenaline was circulating in the head. Under the conditions described above the adrenaline after perfusing the head was returned to the heart. To exclude completely the possibility of a peripheral effect as the cause of the result, a number of experiments was carried out, in which the venous blood returning from the head was temporarily not allowed to enter the heart-lung circuit, but was instead collected into a separate reservoir. To this purpose a cannula was placed in the right subclavian vein, near its origin from the superior cava: by clamping the latter below

the cannula, the venous blood passed into the cannula and through a rubber tube into a beaker kept 10–15 cm. below the level of the heart. This deflection of the course of the blood from the head was only maintained long enough to allow a comparison between the effect of aortic pressure before and after adrenaline.

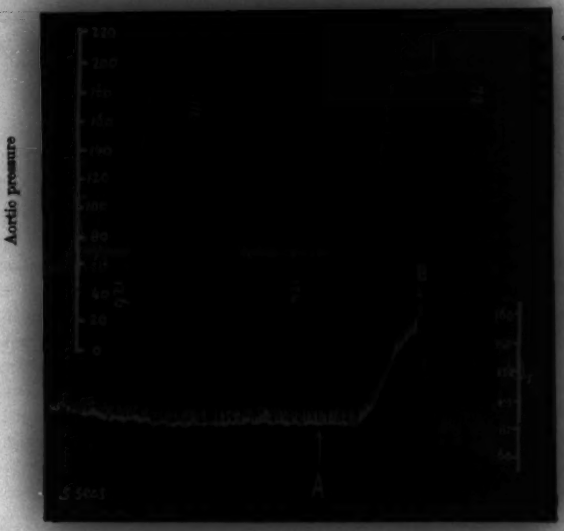

Fig. 4. Dog. 12 kg. Innervated heart-lung preparation. Head (lower tracing) perfused by means of a Dixon pump. At *A* 0·8 c.c. of 1 : 40,000 adrenaline is slowly injected in the tube leading to the pump and head. At *B* the output of the pump perfusing the head is diminished and brought down below the level it had before the injection. Upper tracing: pressure in the aorta. Lower tracing: pressure in the carotids.

Figs. 4 and 5 represent two such experiments. In both, at *A*, a small dose of adrenaline was slowly injected by means of a syringe into the tube leading to the Dixon pump and head. The increase of pressure in the head (see lower tracing) caused by the vaso-constriction from adrenaline was counteracted at *B* by diminishing the output of the pump.

The upper tracing is the pressure record in the aorta; both in the case of Fig. 4 and that of Fig. 5 the effect of adrenaline upon the reflex

slowing of the heart from aortic rise of pressure is well pronounced: in Fig. 4 the difference is of the order of 1–1·5, in Fig. 5 it is of the order of 1–2, these figures representing the usual range in the majority of all the experiments performed.

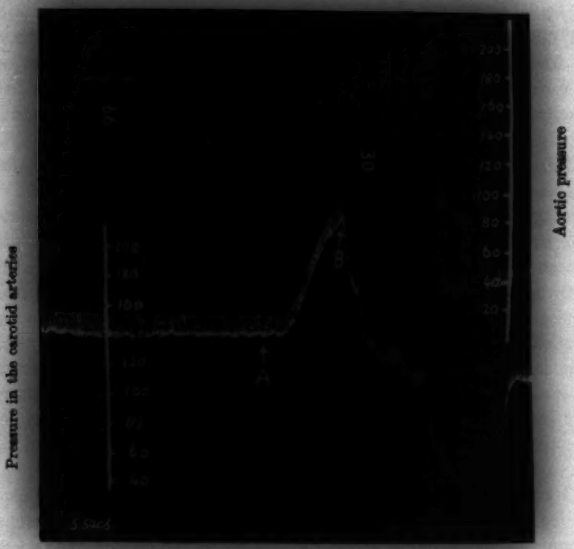

Fig. 5. Dog. 10 kg. Same as in Fig. 4. At *A* 1 c.c. of 1 : 50,000 adrenaline injected into the head circuit. At *B* perfusion pressure in the head artificially lowered.

Conclusion.

It has already been pointed out that the increased reflex response of the heart rate to pressure changes during the action of adrenaline cannot be explained as a result of peripheral sensitization to inhibitory vagus impulses; the experimental conditions employed, moreover, exclude an action upon the peripheral end of the afferent path of the reflex, so that the most likely possibility is that the cardio-inhibitory centres, under the action of adrenaline, become more excitable. This conclusion is not in agreement with that arrived at by C. Heymans [1929] according to whom "l'adrénaline n'a aucune action spécifique, ni directe,

ni indirecte, sur le centre cardio-inhibiteur du chien"; but it would seem that the experimental conditions employed by Heymans (section of the afferent path of the reflex) were not such as to justify that conclusion. The only justifiable conclusion to be drawn from them would be that adrenaline does not actually excite the cardiac vagus centre. The doses used by Heymans, and in the present series of experiments, were very moderate ones, just such as would give in the intact animal a good rise of arterial pressure together with definite slowing of the heart: whether doses much higher than those might excite the vagus centres directly, or better, independently of afferent inhibitory impulses from the depressor and carotid sinus nerves, was not studied.

What is the mechanism through which adrenaline increases the reflex responses of the cardiac centres of the vagus to afferent stimuli? Before entering the discussion of this problem, which anyhow must be left open at the present, it is interesting to note that the results of the present work seem to support a view put forward by G. Viale [1928, 1930], according to whom the vagal tonus is maintained by the presence of adrenaline in the circulating blood. On the other hand, the fact that adrenaline does not directly excite the vagus centres, but simply makes them more responsive to afferent inhibitory impulses, perhaps explains the reason why the injection of adrenaline in an animal with otherwise very low arterial pressure and quick heart rate (low vagal tone) is not so effective in bringing down the heart rate [W. W. Sawitsch and E. N. Speranskaja-Stepanowa, 1927].

According to Anrep and Starling [1925] the effect, upon the cardiac centres, of adrenaline circulating in the head is most probably an indirect one, brought about by changes in the calibre of the vessels in the brain and readjustment of flow and pressure in the different centres. They pointed out in fact that, even if the pressure in the carotids be kept artificially constant, during the action of adrenaline, the pressure in the Circle of Willis and the arteries originating therefrom must be higher than before adrenaline, owing to the peripheral vaso-constriction and slower rate of flow.

It is very unlikely, however, that the variations of pressure in the cerebral arteries are at any rate the only mechanism through which the described effect upon the heart rate is produced, the more so, since, according to experiments of Hering [1927] and Heymans [1930] differences of pressure also of very large magnitude in the cerebral circulation leave the heart rate unaffected, whereas the variations of pressure produced by adrenaline, when the carotid pressure is main-

tained constant, are bound to be of moderate degree. In the experiments described in the first part of this paper, the arterial pressure, at the very time during which adrenaline was displaying its action upon the cardiac vagal centres, was usually low, 80 mm. Hg, or lower, and it is difficult to conceive that it was of such importance for the heart rate. But things are still clearer in experiments such as those of Figs. 4 and 5, in which the cerebral pressure was artificially lowered well below the level it had before the injection of adrenaline, and in which bradycardia still remained. It appears, therefore, that variations of pressure in the Circle of Willis and its branches cannot represent the whole mechanism, and it seems right to say the whole mechanism because I found, in confirmation of Volhard [1930], that if the pressure in the brain circulation is not interfered with but allowed to rise under the action of adrenaline, the slowing of the heart becomes more marked still. Whether here the amount of blood and oxygen supplied at different perfusion pressures plays any part, or whether the effect is due to some sort of sensitization to changes of pressure, which might take place in territories, otherwise irresponsive to the latter, is a question for further study. Also the possibility of increased intracranial pressure cannot be dismissed.

Summary.

1. Adrenaline, injected intravenously in dogs, in moderate doses (about 0·002–0·005 mg. per kg.), enough to cause increase of arterial pressure and marked slowing of the heart, does not seem to have any direct exciting effect upon the cardio-inhibitory centres.

2. Adrenaline administered in these amounts is, nevertheless, not entirely without action on the centres, since it increases the reflex excitability of the cardio-inhibitory centres to afferent impulses from the depressor and the carotid sinus nerves.

3. Adrenaline bradycardia is reflex in origin, and due to the arterial pressure acting upon the sensitive regions of the carotid sinus and of the territory of distribution of the depressor nerves: its degree, however, is conditioned by a state of increased reflex excitability of the cardiac vagus centres caused by the injected adrenaline.

In conclusion I want to thank Prof. C. Lovatt Evans for his advice and criticism. This work was begun at Cambridge during the tenure of the G. H. Lewes Studentship. For the part performed at University College the expenses were defrayed by a grant from the Foulerton Reserve Fund of the Royal Society, for which I am most thankful.

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PHLORRHIZIN DIABETES IN FASTING OR FED HYPOPHYSECTOMIZED DOGS.

By A. BIASOTTI and B. A. HOUSSAY.

(*Institute of Physiology, Faculty of Medicine, Buenos Ayres.*)

We have shown that extirpation of the pituitary gland or of its anterior lobe, or cauterization of the tuber cinereum, prevents or diminishes diabetes following pancreatectomy in the toad. The implantation of the anterior lobe (and to a lesser degree the posterior) causes the onset of a severe diabetes in these operated animals [Houssay and Biasotti, 1931 *a*].

Also in the hypophysectomized toad, or toad without the anterior lobe, phlorrhizin glycosuria is less marked than in the control, the implantation of the anterior lobe causing it to increase [Di Benedetto, 1931].

In hypophysectomized dogs total pancreatectomy usually produces only a mild diabetes as compared with the controls, survival is prolonged (up to 6 months), the weight decreases more slowly, the hyperglycæmia and glycosuria are less, during fasting there is no great destruction of protein, the D : N ratio is low (0·7–1·85) and the basal metabolism is not raised or is raised only very slightly. In dogs with a lesion of the tuber cinereum pancreatic diabetes has the usual rapid development with marked glycosuria [Houssay and Biasotti, 1931 *c*]. The same occurs in thyroidectomized dogs [Yriart, 1930].

Phlorrhizin diabetes in fasting hypophysectomized dogs is characterized by death usually with hypoglycæmia, the glycosuria being only a third of that in the controls, the urinary nitrogen less, and the D : N ratio low. On the other hand, five dogs with lesions of the tuber cinereum had a phlorrhizin diabetes similar to that of the controls [Houssay and Biasotti, 1931 *b*].

A brief summary of the marked metabolic changes occurring in hypophysectomized animals may be found in our previous publications [Houssay, 1931].

Methods.

We have made comparative studies of phlorrhizin diabetes during fasting in thirty-one dogs, seven hypophysectomized, four with the

posterior lobe removed, nine with lesions of the tuber cinereum, six thyroidectomized, and five controls. We have also compared the action of phlorrhizin in four hypophysectomized dogs and four controls on a meat diet, five hypophysectomized and four controls on a sugar diet, and four hypophysectomized and three controls on a fat diet.

Before the experiments the dogs were kept in metabolism cages on a diet of 400–500 g. of meat and the urine was measured daily. After a day of fasting they received intravenously 1 g. of phlorrhizin dissolved in 25 c.c. of 1·2 p.c. sodium bicarbonate solution; on subsequent days 1 g. suspended in 10 c.c. of olive oil was injected under the skin of the flank or abdomen. Kahlbaum's and Grübler's preparations of phlorrhizin were used. Each day the dogs were weighed, the volume of urine measured and determinations made of the urinary glucose (Benedict's method), nitrogen (Folin) and total ketone bodies (van Slyke). The blood sugar was determined (Hagedorn and Jensen) at the beginning and end of the experiment and sometimes every other day.

The fasting dogs could drink water *ad lib.* Those on a meat diet had 300 g. daily of good fresh lean beef, and those on a sugar diet 50 g. of cane sugar. Those on a fat diet received 100 c.c. of an emulsion of oil (600 c.c. of olive oil, 300 c.c. of water and 5 c.c. of 40 p.c. NaOH mixed together, and 48 hours later the emulsified oil decanted). The oil and at times the sugar was given by stomach tube.

The operations on the pituitary were performed by the left temporal route and verified at autopsy and by histological examination. The lesion of the tuber cinereum was done by galvano-cautery from the pituitary stalk to the posterior limit of the corpora mammillaria.

The starvation experiments lasted 5 days in thyroidectomized dogs and dogs deprived of the posterior lobe; in ten hypophysectomized dogs taken in the order in which they are presented in the tables 3, 5, 6, 6, 3, 3, 3 days respectively. They lasted 4, 3, 4, 4, 4, 6, 6, 6, 6 days in those with the tuber lesions and 7, 6, 5, 7, 3 days in the controls.

On meat diet the experiments lasted 7 days in the hypophysectomized dogs and controls; on sugar 6 days; on fat 5 days in the controls and in the hypophysectomized only 3, 3, 3 and 4 days owing to the onset of a severe or fatal hypoglycæmia.

The time elapsing after the operation and before the administration of the phlorrhizin was, in the order of the tables of this paper, in fasting experiments, after hypophysectomy 299, 260, 73, 194, 51, 120, 105 days; after lesions of the tuber cinereum 20, 26, 36, 47, 46, 45, 44, 44, 46 days; after thyroidectomy 55, 55, 55, 55, 137, 137 days; after removal of the

posterior lobe 97, 61, 57, 57 days; after the hypophysectomy for experiments on a meat diet 104, 51, 102, 342 days; on sugar 151, 136, 94, 143, 62 days; on fat 346, 35, 77, 74 days.

Blood-sugar results.

Hypophysectomized dogs readily showed severe hypoglycæmia under various conditions:

(1) At times spontaneously.

(2) Always during prolonged fasting.

(3) Frequently after the injection of adrenaline during fasting.

(4) During the action of insulin [Houssay and Magenta, 1925–7–9].

(5) In some cases after total extirpation of the pancreas.

(6) Always after injection of phlorrhizin [Houssay and Biasotti, 1931 *b*].

Table I. Dogs under phlorrhizin. Blood sugar mg./100 c.c.

Fasting.

	Hypophysectomized		Tuberal lesions		Without posterior lobe		Thyroidectomized		Controls	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	109	56	92	81	96	66	110	70	104	70
	106	70	108	118	84	98	118	90	110	114
	88	70	111	92	88	78	102	104	114	117
	106	72	120	95	82	66	120	80	124	118
	81	67	102	81			96	88		
			94	74			104	92		
			98	77						
			110	89						
Mean	98	67	104	88	87	77	108	87	113	104

Fed.

	Meat				Sugar				Fat			
	Hypophysectomized		Control		Hypophysectomized		Control		Hypophysectomized		Control	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	113	104	92	84	102	90	96	104	88	57	114	127
	86	88	99	98	88	80	116	98	90	48	130	128
	118	94	104	90	80	86	98	106	91	58	92	106
	92	86	112	98	108	92	110	114	84	62		
					96	84						
Mean	102	93	101	92	94	86	105	105	88	56	112	120

In fifteen out of seventeen hypophysectomized dogs injected with phlorrhizin there was a severe or fatal hypoglycæmia. Some of these animals were saved by repeated intake of sugar and later of food, when the condition had not been present long. Of the fasting dogs injected

with phlorrhizin only the hypophysectomized showed intense hypoglycæmia and severe symptoms, several of them dying. These symptoms, appearing usually during the third or fourth day when the blood sugar had dropped to 0·07 p.c. were weakness, tremors, inability to stand, salivation, opisthotonus, stepping movements, partial or general convulsions, lowered temperature, slow respiration, flaccidity, and finally death. No such symptoms occurred during fasting in the controls, nor in any of the fasting animals which had had operations other than hypophysectomy.

Feeding with meat or sugar maintained the blood sugar normal and prevented symptoms in the hypophysectomized dogs. On a fat diet there was severe hypoglycæmia in the four hypophysectomized animals with death on the third or fourth day, but no hypoglycæmia occurred in the controls in 6 days.

Glycosuria.

During starvation the maximum elimination of sugar occurred on the third and fourth days, the D : N ratio being between 2·61 and 3·7 in the controls.

TABLE II. Dogs under phlorrhizin: glucose eliminated in g. per kg. per diem. Average for each dog of each group.

	Fasting.				
	Hypophysectomized	Tuberal lesion	Without posterior lobe	Thyroidectomized	Controls
	0·51	1·47	2·63	1·70	2·73
	0·70	1·15	2·87	2·45	1·84
	0·81	1·73	2·10	2·02	2·64
	0·91	0·90	1·64	2·00	1·25
	0·80	1·63		1·70	1·55
	0·42	1·70		1·61	
	0·66	1·70			
		1·92			
		1·51			
Mean	0·68	1·54	2·31	1·91	2·00

	Fed.					
	Meat		Sugar		Fat	
	Hypophysectomized	Control	Hypophysectomized	Control	Hypophysectomized	Control
	3·61	4·02	1·98	5·08	0·57	2·07
	3·39	4·64	3·60	4·34	0·57	2·98
	3·20	3·91	2·67	3·19	1·04	2·62
	3·00	4·33	2·40	4·33	1·10	
			2·17			
Mean	3·30	4·22	2·56	4·23	0·82	2·55

As can be seen in Table II, among the fasting animals treated with phlorrhizin the controls, the thyroidectomized and those deprived of the posterior lobe showed marked glycosuria, while the elimination of sugar in those which had been hypophysectomized was only a third of that in the controls. In those with lesion of the tuber it was fairly high (1·54 g.) compared with that of the hypophysectomized (0·68 g.), but somewhat less than that of the controls (2·00 g.).

On meat and on sugar diets the daily glucose elimination per kg. in the hypophysectomized dogs treated with phlorrhizin was respectively 23 p.c. and 21 p.c. less than in the controls. On fat diet the elimination was comparable to that in fasting, *i.e.* the hypophysectomized eliminated 67 p.c. less sugar than the controls.

Diuresis was proportional to the quantity of sugar eliminated, the concentration of glucose being similar in all cases (5–8 p.c.). Nevertheless some of the dogs with lesion of the tuber cinereum showed polyuria dating from before the injections.

Urinary nitrogen elimination.

It can be seen from Table III that the nitrogen elimination in fasting dogs without phlorrhizin (7–10 days) was less in the hypophysectomized and thyroidectomized (0·25 g. per kg. per diem) than in the controls or

TABLE III. Urinary nitrogen in g. per kg. per diem. Average for each group.

	17 dogs hypophysectomized	5 dogs tuberal lesion	4 dogs thyroidectomized	10 dogs controls
Fasting without phlorrhizin	0·25	0·36	0·25	0·36
Fasting with phlorrhizin	0·36	0·66	0·63	0·80
Total increase	0·11	0·30	0·38	0·44
Increase p.c.	44	83	152	122

those with lesions of the tuber (0·36 g.). Phlorrhizin administered during fasting caused a very slight absolute increase in nitrogen elimination in the hypophysectomized (0·11 g.), a large increase in the controls (0·44 g.), slightly less in the thyroidectomized (0·38 g.), and less still in those with tuber lesion (0·30 g.). The proportional increase was larger in the thyroidectomized and controls, much less in the hypophysectomized and intermediate in those with lesions of the tuber.

On comparing the five groups of fasting dogs under phlorrhizin (Table IV), the greatest daily excretion of nitrogen per kg. occurred in dogs without the posterior lobe and in the controls, while in the hypo-

TABLE IV. Dogs under phlorrhizin: Urinary nitrogen in g. per kg. per diem. Average for each dog of each group.

Fasting.

	Hypophysectomized	Tuberal lesion	Without posterior lobe	Thyroidectomized	Control
	0·51	0·53	0·71	0·62	0·91
	0·33	0·44	1·24	0·75	0·78
	0·31	0·99	1·00	0·61	0·89
	0·34	0·68	0·95	0·60	0·52
	0·19	0·60		0·51	0·77
	0·21	0·52		0·51	
	0·62	0·69			
		0·60			
Mean	0·36	0·63	0·97	0·60	0·77

Fed.

	Meat		Sugar		Fat	
	Hypophysectomized	Control	Hypophysectomized	Control	Hypophysectomized	Control
	1·60	1·65	0·16	0·69	0·26	0·59
	1·47	1·62	0·41	0·62	0·30	0·88
	1·19	1·42	0·35	1·04	0·33	0·71
	1·29	1·57	0·30	0·70	0·46	
			0·28			
Mean	1·37	1·56	0·30	0·76	0·33	0·73

physectomized it was much less (45 p.c. of that of the controls), and that in dogs with lesions of the tuber or excision of the thyroid it was intermediate.

On fat and sugar diets there was practically the same nitrogen elimination as in fasting, both in the hypophysectomized dogs and the controls. On a meat diet there was naturally a large increase, reaching 1·37 g. N per kg. daily in the hypophysectomized and 1·56 g. in the controls. As both groups received 9·1 g. N daily and the initial and final weights were 7·2 and 7·1 kg. in the hypophysectomized and 8·1 and 7·2 kg. in the controls it can be calculated that the first received 1·26 g. per kg. N daily and eliminated 1·38 g. and the controls received 1·12 g. and eliminated 1·56 g.; that is to say, the hypophysectomized lost 0·12 g. of their own nitrogen per kg. per diem and the controls 0·44 g., which explains in great part the decrease in weight in the latter. The meat given produced a certain saving of nitrogen in both groups, for if we add the amount eliminated during fasting to that given in the meat we get 1·91 g. for the controls and 1·62 g. for the hypophysectomized, whereas they actually eliminated 1·54 and 1·38 g. respectively, *i.e.* 0·37 and 0·24 g. less.

TABLE V. Initial weights (*a*), and final weights (*b*), of dogs treated with phlorrhizin.

Fasting.

	Hypophysectomized		With lesion of tuber		Without posterior lobe		Thyroidectomized		Controls	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
	16	15·3	10·2	8·9	6·5	5·3	8·3	7·6	14·5	12·3
	17·9	16·0			11·2	9·0	7·1	6·1	10·7	8·8
	9·5	8·8	7·3	6·8	8·0	6·9	8·1	6·5	9·5	8·2
	9·1	8·5	6·3	5·0	7·6	7·0	9·5	8·4		
	9·0	8·7	5·6	4·8			10·2	9·1	12·2	10·8
	10·8	10·1	9·0	8·2			7·4	7·1	9·4	8·0
	9·4	8·4	8·4	7·5						
			9·0	8·4						
			8·2	7·0						
Mean	11·7	10·8	8·0	7·0	8·3	7·0	8·4	7·5	11·2	9·6

Fed.

	Meat				Sugar				Fat			
	Hypophysectomized		Control		Hypophysectomized		Control		Hypophysectomized		Control	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
	6·9	6·5	6·6	6·6	6·0	5·7	7·5	7·1	12·0	11·6	9·5	8·5
	6·1	6·2	8·3	7·3	6·2	5·5	13·6	12·0	6·8	6·6	8·2	7·1
	8·8	8·5	10·2	9·0	13·8	12·3	8·0	7·0	6·8	6·7	9·0	7·8
	9·3	9·4	7·3	6·8	9·4	8·1			6·5	6·0		
					11·5	10·7						
Mean	7·7	7·6	8·1	7·4	9·3	8·4	9·7	8·7	8·0	7·7	8·9	7·8

Weight.

The administration of phlorrhizin during fasting caused a smaller loss of weight in the hypophysectomized (11 p.c.) than in the controls or in those with lesions of the tuber (15–16 p.c.), and the loss was intermediate in the thyroidectomized (13 p.c.). This difference was maintained on a sugar or fat diet; on meat diet the loss in the hypophysectomized was appreciably less than in the controls, in which the loss was less (11 p.c.) than during fasting. Apparently the loss of weight is related to the destruction of the tissues and body protein.

D : N ratio.

With phlorrhizin during fasting the average D : N ratio for each group was: 2·97 in the controls, 2·50 in those without the posterior lobe, 3·17 in the thyroidectomized and 3·50 in those with lesions of the tuber. In the hypophysectomized the ratio was very low (1·62) with the peculiarity that there was no rise on the third and fourth days as observed

in the other groups. On a fat diet the ratio rose in the hypophysectomized to 2·45 and to 3·55 in the controls. On a meat diet the ratio was similar in both groups, being 2·38 in the hypophysectomized and 2·80 in the controls.

Acidosis.

Dr Rietti has studied the acetonuria in pacreatic and in phlorrhizin diabetes in fasting and fed animals. These experiments, which are being published separately, show that this is always lower in the hypophysectomized dogs.

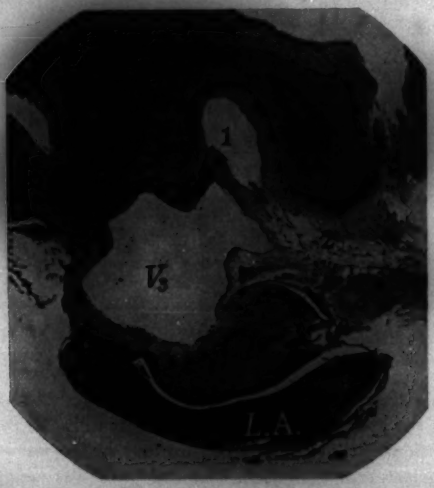

Fig. 1. Hypophyseo-tuberal region in a dog in which the tuber cinereum was cauterized. Sagittal section. *L.A.* anterior lobe of the hypophysis; *L.P.* posterior lobe of the hypophysis; V_3, third ventricle; 1, cystic cavity; 2, lacunar zone in the damaged region; 3, fibrous scar.

Histological examination.

Serial sections of the pituitary region were cut in all dogs which had had operations on the pituitary or tuber cinereum, the histological examination being undertaken with the technical assistance of Drs Lascano-Gonzalez and Sanmartino. In all the hypophysectomized animals the anterior lobe was missing, but there were always remains of the pars tuberalis adhering to the infundibulum, and in a few cases some

fragments of pars intermedia. In those dogs with lesions of the tuber there were necrotic remains or loss of substance, 4 or 5 mm. in length from the pituitary stalk to the posterior limits of the corpora mammillaria (destroyed), 2 to 4 mm. in depth and 3 to 4 mm. in breadth. In more than half the posterior lobe was destroyed; the anterior lobe was preserved complete or almost complete with its several types of cells.

Discussion.

We have observed that hypophysectomized dogs readily develop hypoglycæmia during fasting or under the influence of certain substances, especially phlorrhizin. This hypoglycæmia must be due to the absence of the anterior lobe, because it is not observed in dogs deprived of the posterior lobe or thyroid or with lesions of the tuber. This hypoglycæmia and the fatal symptoms accompanying it do not occur if the dogs receive meat or sugar, but they are not prevented by fat.

The glycosuria set up by phlorrhizin in hypophysectomized fasting dogs is much less than in the other groups, which must be attributed to the anterior lobe deficiency. A fat diet does not modify it. The intake of meat increases the glycosuria although it is slightly less in the hypophysectomized than in the controls. It seems then that with phlorrhizin hypophysectomized dogs produce little glucose during starvation with no exogenous protein metabolism, while the production is larger when they are given protein in sufficient amount. This can be compared with the observation by Braier (1931) that hypophysectomized dogs have a lower endogenous protein metabolism, as shown by the following:

(1) On a meat diet they eliminate less creatinine than controls.

(2) During complete fasting or protein fast they eliminate 30–40 p.c. less nitrogen and 35 to 40 p.c. less creatinine than controls.

(3) When *B. coli* vaccine is injected the protein catabolism increases less.

(4) In diabetes due to total pancreatectomy their protein catabolism during fasting is less than if the pancreas is excised with the pituitary *in situ*; if they are fed they also live longer because of less wasting of their tissues.

It is reasonable to suppose that the fundamental change in hypophysectomized dogs is in their limited capacity to form glucose from the proteins of their tissues, in which case an important rôle of the anterior lobe would be that of regulator of endogenous protein metabolism.

It seems that when treated with phlorrhizin hypophysectomized animals are able to conserve or transform part of the sugar or its pre-

cursors formed from protein. This explains their very low D : N ratio when fasting and the low ratio when on a meat diet. In a previous paper we have shown that when after excision of the pancreas their blood sugar was not very high, hypophysectomized dogs could retain part of the sugar administered and their respiratory quotient rose.

The tuber lesion somewhat diminishes phlorrhizin glycosuria and nitrogen elimination during fasting. This can be attributed to a slight functional disturbance of the anterior pituitary lobe as shown by similar observation in the toad already quoted, especially if we remember that the tuber sends nerve fibres to this gland and that this in its turn seems to influence the tuber by a secretion which reaches it both through the stalk and by the blood stream.

Thyroidectomy does not affect phlorrhizin glycosuria in fasting dogs, reduces the nitrogenous elimination in fasting without phlorrhizin and reduces slightly that observed in fasting dogs under phlorrhizin. But the alterations caused by hypophysectomy are incomparably more pronounced than those following thyroidectomy.

Summary.

1. In hypophysectomized fasting dogs phlorrhizin glycosuria is much less marked than in controls, in thyroidectomized dogs and in those without the posterior lobe of the pituitary. In the hypophysectomized there is an early fatal hypoglycæmia which does not occur in the other groups.

2. On a meat diet the glycosuria is much less in hypophysectomized dogs than in controls. Hypoglycæmia and death are prevented by feeding meat or sugar but are not prevented by fat.

3. The nitrogenous elimination in fasting is lower after hypophysectomy and after thyroidectomy than in the controls or after tuber lesions. The fasting elimination after phlorrhizin is less in the hypophysectomized than in the thyroidectomized, in those with tuber lesions, in the controls and in those without the posterior lobe. On a sugar or fat diet the fasting figures are only slightly modified, but on a meat diet the nitrogenous elimination of the hypophysectomized is nearer that of the controls.

4. In the hypophysectomized dogs the loss in weight is always more gradual. The D : N ratio and degree of ketosis are less.

5. It appears that the capacity to form sugar at the expense of the endogenous protein is greatly diminished in hypophysectomized dogs.

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KETOSIS IN THE PANCREATIC AND PHLORRHIZIN DIABETES OF HYPOPHYSECTOMIZED DOGS.

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In the hypophysectomized toad total extirpation of the pancreas either does not produce diabetes or causes merely a slight rise in the blood sugar. The implantation of the anterior lobe of the pituitary definitely produces or increases the diabetes of the pancreatectomized toad, whether previously hypophysectomized or totally decerebrated [Houssay and Biasotti, 1931 *a*].

Work done by Houssay and Biasotti [1931 *b* and *c*] shows that the diabetes caused by pancreatectomy or by injection of phlorrhizin is considerably diminished in dogs which have previously been subjected to hypophysectomy. This diminution of diabetes is shown particularly as regards the glycosuria, blood sugar, time of survival and D : N ratio. The present data as to the excretion of ketones in the diabetic dog indicate also a decrease in the severity of the diabetes in hypophysectomized animals. They also show that the anterior lobe of the pituitary is concerned in the ability of the organism to form ketone bodies.

Animals and Method. 74 dogs were used. The samples of urine were taken almost entirely from the animals experimented on by Drs Houssay and Biasotti. The operative methods are described in the work quoted, from which the figures relating to the sugar in blood and urine given in Tables I to III have been taken.

The method of determination of the total ketone bodies was that of van Slyke [1917] modified by using only 10 c.c. of urine instead of 25 c.c., reducing in equal ratio the reagents used in the first part of the method.

Results.

1. *Ketosis in pancreatectomized dogs.* Under this heading we include a summary of the data already published [1931] with the addition of the results obtained in six other dogs.

Examining Table I, it is seen that in the controls there is an average daily excretion of 76 mg. of ketone bodies per kg.; this is low compared

TABLE I. Ketosis in pancreatic diabetes in dogs.

	With hypophysis excised (9 dogs)	Controls (6 dogs)	With tuber cauterized (5 dogs)
Daily average:			
Ketosis (acetone in mg./kg.)	21	76	31
Glycosuria (sugar in g./kg.)	1·05	3·00	2·04
Blood sugar (average mg./100 c.c.)	209	380	298

with the values obtained by Hedon [1930], who found on suspending the administration of insulin to a pancreatectomized dog the ketone bodies in 24 hours amounted to more than 0·5 g. per kg. per diem. MacLeod [1928] quotes data of Chaikoff, who found about 0·3 g. of ketone bodies per kg. excreted daily from the third to the sixth day after deprivation of insulin and food in pancreatectomized dogs.

Our dogs received insulin in the post-operative period during healing of the wounds. Each day they had 100 g. of fresh bovine pancreas and about 300 g. of meat. During the days when the observations recorded in Table I were made, they did not have insulin. All our determinations give lower ketone values than those obtained by the workers quoted, which must be attributed to the different treatment of the animals.

The average weight of the hypophysectomized dogs was 8·3 kg., the smallest 6·4 kg., the heaviest 11·9 kg.; that of the controls ranged from 6 to 15 kg., with average 9·3 kg.; the dogs with cauterized tuber were a little lighter (average 5·7 kg.). The pituitary had been removed several weeks or even months previously. The observations on the urine extended over several days, in three cases over three weeks or more.

TABLE II. Ketosis in phlorrhizin diabetes in fasting dogs.

	With hypophysis excised (6 dogs)	Controls (6 dogs)	With tuber cauterized (7 dogs)	With thyroid excised (6 dogs)	With post-lobe removed (4 dogs)
Daily average:					
Ketosis (acetone in mg./kg.)	5	88	120	123	116
Glycosuria (sugar in g./kg.)	0·81	1·71	1·38	1·91	2·31
Blood sugar (average mg./100 c.c.)	70	89	92	97	80

Of the nine hypophysectomized dogs, which never received insulin, five excreted ketone bodies equivalent to 13 mg./kg. or less in 24 hours: two of the remaining four excreted 25 and 28 mg., which is less than the smallest amount obtained from any of the controls, and the other two 44 and 54 mg. In these latter four dogs the diabetes came on with great rapidity and intensity and there was a high blood sugar; the one that

gave 44 mg. had the highest degree of glycosuria of all (2·83 g./kg.). In three of the dogs with very low degree of ketosis the diabetes was slight with but little hyperglycæmia; from time to time in these animals hypoglycæmic attacks came on which were of some severity and finally caused death. The average ketone excretion for the whole group was only 28 p.c. of that for the controls, and the average blood sugar and average urinary sugar were, as shown in Table I, conspicuously lower than in the controls.

In the hypophysectomized dogs the total ketone elimination varied to some extent with the blood sugar and with the urinary elimination of sugar. Pancreatectomized dogs with lesion of the tuber cinereum (produced by galvano-cauterization from the pituitary stalk to the posterior limits of the corpora mammillaria) showed an average ketone elimination intermediate between that of hypophysectomized and of control animals.

A somewhat similar effect of lesion of the tuber cinereum has been observed by Houssay and Biasotti in the glycæmia of phlorrhizin diabetes, which seems to indicate a slight inhibition of the anterior pituitary. Three results are lower than the average for the hypophysectomized. The blood sugar is higher than in the hypophysectomized dogs.

2. *Ketosis in phlorrhizin diabetes during fasting.* Most of the dogs used were those experimented on by Drs Houssay and Biasotti, who showed that the diabetes due to phlorrhizin injection was diminished in hypophysectomized dogs. Before the experiments the dogs were kept in separate metabolism cages and received daily 400 to 500 g. of beef. At the beginning of the experiment they were starved, but were allowed water *ad lib.*; that day they received intravenously 1 g. of Kahlbaum's phlorrhizin dissolved in 25 c.c. of 1·2 p.c. sodium bicarbonate solution and on each succeeding day 1 g. of phlorrhizin suspended in 20 c.c. of olive oil was injected under the skin of abdomen or flank. Phlorrhizin was injected into controls and into dogs which had been hypophysectomized more than two weeks and up to as much as nine weeks before, or thyroidectomized eight weeks or more previously, or had lesions of the tuber cinereum which were three weeks or more old or had been deprived of the posterior lobe for two or three months. The observations on the urine of each day extended generally over four or five days, but sometimes over a period longer than that.

The results are shown in Table II, from which it can be seen that the hypophysectomized eliminate much less ketone bodies (5 mg./kg. in 24 hrs.) than the other animals (88–123 mg./kg.).

They also have a lower blood sugar and less glycosuria. There is

probably less production of sugar as the respiratory quotient and basal metabolism do not show an increase in the consumption of carbohydrate according to the experiments of Houssay and Biasotti.

3. *Ketosis in phlorrhizin diabetes in fed animals.* The total ketone bodies present in the urine of twenty-five dogs was determined, completing the work of Drs Houssay and Biasotti.

The food daily received by the dogs is shown in Table III. The phlorrhizin was given in the same way and amounts as described for the fasting dogs.

TABLE III. Ketosis in phlorrhizin diabetes in (*a*) hypophysectomized, (*b*) control dogs.

	Fed with					
	Meat, 300 g. daily		Sugar, 50 g. daily		Fat, 100 c.c. olive oil emulsion daily	
	(*a*) 5 dogs	(*b*) 4 dogs	(*a*) 5 dogs	(*b*) 4 dogs	(*a*) 4 dogs	(*b*) 3 dogs
Daily average:						
Ketosis (acetone in mg./kg.)	12	56	18	35	11	74
Glycosuria (sugar in g./kg.)	3·3	4·2	2·6	4·2	0·8	2·6
Blood sugar (average mg./100 c.c.)	92	95	89	104	56	114

On the whole, food increases the ketone elimination in hypophysectomized animals and decreases it in the controls (see Table III). In both groups the blood sugar and glycosuria are raised. The hypophysectomized always eliminate much less ketone bodies than the controls on a similar diet.

Meat diet. The hypophysectomized dogs show only slight increase in ketosis (from 5 mg. in fasting to 12 mg.) in spite of great increase in the glycosuria; the controls show slight diminution (88 mg. in fasting and 56 mg. on meat diet).

Sugar diet. The controls show much lower ketone elimination (35 mg.) than in fasting (88 mg.) in accordance with the known anti-ketogenic action of glucose. This action apparently fails in the hypophysectomized where the ketone elimination is more than three times that in fasting (18 mg., compared with 5 mg.).

Fat diet. The controls eliminate similar amounts (88 mg. in fasting and 74 mg. on fat diet). The hypophysectomized show a slight increase (from 5 to 11 mg.), but it is not possible to draw definite conclusions owing to the brevity of the observations as hypophysectomized dogs on a fat diet die rapidly in hypoglycæmia (on the second, third and fifth days), while they survive on meat or sugar.

General conclusions.

1. Ketone elimination is notably less (1 : 3·6) in the pancreatic diabetes of hypophysectomized dogs than in the controls. It bears a relationship to the level of the blood sugar. It is intermediate in animals with lesions of the tuber cinereum.

2. The ketone elimination is much less (1 : 17) in the phlorrhizin diabetes of hypophysectomized fasting dogs than in the normal controls; in thyroidectomized animals, in those without the posterior pituitary lobe, and those with lesions of the tuber cinereum ketone elimination is greater than in the controls.

3. In hypophysectomized dogs on meat, sugar or fat diets the ketone elimination in phlorrhizin diabetes is always less than in the corresponding controls.

4. The intake of sugar diminishes the ketone elimination in the controls, but in the hypophysectomized there is a slight rise.

5. The ketone elimination of hypophysectomized fed dogs under phlorrhizin does not increase as much as does the glycosuria during meat intake.

6. Pituitary insufficiency always diminishes the urinary elimination of ketone bodies.

This work was suggested by Professor Houssay in connection with his researches on diabetes in hypophysectomized dogs. He gave me the opportunity of carrying it out and constant help and valuable suggestions for which I am deeply grateful.

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THE BLOOD CHOLESTEROL IN ANÆSTHESIA.

By A. C. GHOSE, *Research Scholar.*

(*Department of Surgery, Lucknow University.*)

Many observations on the cholesterol content of the blood have been made in animals under various conditions. The effect of pregnancy [Chamberlain, 1929], of diet [Bloor, 1932], of injections of cholesterol [Chamberlain, 1928] has been studied and also the relation between it and the fat and lecithine in the blood [Bloor, 1921]. That anæsthesia also affects the blood cholesterol index has been appreciated; Duccheschi [see Gray, 1930] found an increase in the cholesterol in the serum of dogs after repeated administration of chloroform, and Gray [1930] determined the effect in rabbits of repeated administration of chloroform, urethane and paraldehyde. He found the amount of cholesterol in the blood much above normal after chloroform anæsthesia.

Certain experiments to be carried out on rabbits in which the use of chloroform, ether and urethane anæsthesia is necessary, made it desirable to determine the effect of these anæsthetics on the amount of cholesterol in the blood. Other tissues, such as the brain, spleen, liver, suprarenals, gall bladder, etc., are being similarly examined with a view to obtaining light on the physiological and pathological metabolism of cholesterol; but the results with these tissues are reserved for future publication.

Methods.

Anæsthesia was produced in albino rabbits by inhalation of chloroform and ether by the open method. Anæsthesia was induced very slowly and maintained for 1 hour; and then the animals were killed by an overdose of the anæsthetic.

Urethane dissolved in water was given by injection into the peritoneum; the anæsthetic dose given was 0·8 g. per kg. body weight. After 1 hour another dose was given to kill the animal.

The cholesterol content of the whole blood was determined in the alcohol-ether extract as prepared by the method of Bloor [1916] using the Liebermann-Burchard reaction.

Experimental results.

Blood cholesterol in normal rabbits. The normal cholesterol content of the whole blood was found to range between 56 and 105 mg. per 100 c.c., the average value being 80 mg. The average amount of cholesterol in the serum was 48 mg. per 100 c.c. and ranged from 23 to 65 mg. The average values were calculated from the results obtained in 36 rabbits. The results of Grigaut and his co-workers, of Weidman and Suderman, Clarkson-Newburgh and Harnes, as quoted by Gray [1930], are in good agreement with those obtained by him and also by me.

Table I. Chloroform. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
56	65	—	88
57	72	—	122
58	65	—	96
9	84	—	112
10	82	—	130
11	80	110	118
59	75	105	120
60	80	95	134
61	85	112	156
Average	76·4	105·5	119·5

The effect of chloroform anæsthesia (Table I). The blood cholesterol was determined in nine rabbits before anæsthesia and after death from chloroform. In four of them it was also determined during the anæsthesia. There was a slight but definite rise (average 23 mg.) in the blood cholesterol index during the anæsthesia, while after the fatal dose the increase was greater (average 43 mg. per 100 c.c.).

Table II. Ether. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
1	76	—	115
2	62	—	130
3	65	—	125
4	78	95	105
5	85	100	120
6	82	85	94
7	80	90	114
Average	75·4	92·5	114·7

The effect of ether anæsthesia (Table II). Seven rabbits were used. The index was found to be raised after death from ether, significantly in all but one, but on the average from all seven animals by 39 mg., a result similar to that obtained after chloroform. Robinson [1929] found in

cats no change in the blood cholesterol after ether anæsthesia or, if any, a tendency for it to fall. But Mahler [1926] found that in man after ether anæsthesia of an hour's duration the amount of cholesterol in the whole blood always rose.

TABLE III. Urethane. Blood cholesterol in mg./100 c.c.

Rabbit No.	Before anæsthesia	During anæsthesia	After death
62	76	—	95
63	80	—	84
64	75	86	92
65	68	72	100
66	84	98	128
Average	76·6	83·3	99·8

The effect of urethane anæsthesia (Table III). This type of anæsthesia was used in five rabbits. In four of them there was no increase in the blood cholesterol, while in one, No. 66, it rose from 84 to 128 mg. per 100 c.c. During the anæsthesia the changes were not significant.

SUMMARY AND CONCLUSIONS.

An increase in the blood cholesterol index is found after a dose of chloroform or ether sufficient to cause death. During the course of anæsthesia the increase of the blood cholesterol is found to be no more than 20–25 mg. per 100 c.c. After death from urethane no increase in the blood cholesterol was found except in one case, which shows that there is a possibility of the blood cholesterol being increased.

In conclusion I thank Prof. R. N. Bhatia for the constant interest in the work.

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THE METABOLISM OF LACTOSE.

Part II. The blood sugar during lactation.

By L. B. WINTER.

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From experiments described in the previous paper [Winter, 1931] the conclusion was drawn that, by relying only on estimations of reducing substances in the urine, the problem of the utilization of lactose by the tissues could not be satisfactorily settled. The present work was undertaken to discover whether lactose is a threshold substance in the blood. Best [1918] obtained from the blood of the cow a phenyl-osazone which he believed to be that of lactose, but the method by which the sugar was extracted was elaborate and not free from objection in that the blood was boiled with acid before and after removal of the proteins. The matter is discussed fully by Grevenstuk [1929].

Experimental.

The subjects from whom the blood was obtained were healthy nursing women, 3 to 5 days after parturition. Only those cases were chosen in which lactose was present in the urine: the amount was unimportant since, in the case of threshold substances, for any of the substance to be excreted in the urine, the amount in the blood must be above the threshold level. The blood was obtained by venous puncture, and defibrinated as collected: the blood sugar was extracted as described for rabbit blood [Winter, 1930]. Since it was undesirable that any considerable amount of blood should be drawn from a single individual, it was necessary to combine samples. Each batch of blood was therefore brought as quickly as possible to the stage of the dry lead precipitate, at which the sugar can be preserved indefinitely. When sufficient of the lead compound was available to give a satisfactory yield of sugar, the remaining stages were carried through.

The evidence obtainable from osazones derived from sugar mixtures was not likely to be of service; the objective therefore was the isolation in the crystalline condition of any lactose which might be present. When

glucose and lactose are in a syrup together with impurities, it is difficult to separate them; when crystalline it is easy to make use of the difference in their solubilities in methyl alcohol. After crystallization, the blood sugar was extracted with cold methyl alcohol; the residue should contain an increased proportion of lactose were this sugar present, and this would be indicated by the residue having a melting point above that of glucose and a specific rotatory power significantly below it.

The amount of sugar yielded by 271 c.c. blood was 99 mg., this by extraction with methyl alcohol was reduced to 47 mg. The melting point was 138–140° and the specimen showed $[\alpha]_{5461} = +110°$ with a final value of + 60°. In another experiment 103 mg. sugar were obtained from 237 c.c. blood; after extraction 26 mg. remained with M.P. 138–140°, and initial and final rotations of + 115° and + 67° respectively. No evidence of the presence of lactose was obtained by fractional crystallization. The sugar syrup from 158 c.c. blood was drained on a porous tile when only partially crystalline; lactose being the less soluble sugar might be expected to crystallize before the glucose; 25 mg. of solid were obtained and this was further reduced by extraction with alcohol to 12 mg., M.P. 144–145°, $[\alpha]_{5461} = +108°$, falling to + 56° after 18 hours.

An increase in the reducing power of the sugar mixture after boiling with dilute acid would not be proof of the presence of lactose, but identical values before and after hydrolysis would definitely exclude any disaccharide. Two such experiments were performed, the reducing power being estimated by the Wood-Ost method, and the results showed that no trace of lactose could be present. 107 c.c. blood yielded 40 mg. total sugar; the dry residue after extraction with methyl alcohol weighed 27 mg. and melted at 138–140°. 25 mg. were dissolved in 4·56 c.c. of *N*/5 HCl in a small tube, and 2 c.c. of the solution required 3·5 c.c. permanganate of such strength that the calculated glucose content was 10·5 mg., while the actual solid content was 10·9 mg. The tube was weighed, refluxed for 1¾ hours, and re-weighed. A correction was made for the concentration due to the drop of water in the condenser. On titration 2 c.c. required 3·55 c.c. of permanganate (corrected).

In the second experiment 80 mg. of sugar were obtained from 155 c.c. blood. The residue after extraction (15·5 mg., M.P. 135–138°) was dissolved in 4·4 c.c. of acid. 2 c.c. required 1·95 c.c. of permanganate before and 1·85 c.c. (corrected) after hydrolysis. By working up the methyl alcohol solutions α glucose was obtained without difficulty. M.P. 145–146°. 17·6 mg. in 1·615 g. water ($c = 1·07$) showed $[\alpha]^{13}_{5461} = +120°$ with a final value of + 61°.

Micro analyses (by Dr Ing. A. Schoeller, Berlin): C 39·87, 39·99; H 6·54, 6·59; $C_6H_{12}O_6$ requires C 40·00, H 6·66.

During the course of further work it was seen that the blood sugar of lactating women often yielded two distinct types of crystals. Although lactose had been shown to be absent, it was possible that traces of another sugar might be present, the most likely being galactose. The initial rotations of the residues after extraction with methyl alcohol did in fact support this hypothesis, for the values lay near to that for α glucose, although in previous work two recrystallizations were found to be necessary in order to obtain pure α glucose from rabbit blood. Galactose is less soluble than glucose, and would tend to be left in the residue after extraction of the dry mass with alcohol. The total sugar from 440 c.c. blood taken from four cases 4 days after parturition was extracted with cold methyl alcohol, and the residue was recrystallized from the same solvent. Two fractions were obtained.

Fraction 1: 32 mg. M.P. indefinite, 140–152°. The initial and final rotations were + 138° and + 77° respectively. C 39·91, 40·07, H 6·73, 6·75.

Fraction 2: 9 mg., melted gradually between 140° and 150°. $[\alpha]_{5461} = + 146°$, falling to + 83° after 18 hours.

The high rotations, initial and final, and the raised though indefinite melting points in this experiment, might be accounted for by the presence of a small amount of impurity. The elementary analysis of fraction 1, however, was that required for $C_6H_{12}O_6$: a small quantity of galactose whose specific rotation and melting point are + 170° and 168° respectively could account for the discrepancy.

1·13 g. of sugar was accumulated, taken from twenty-five cases at the same stage of lactation, 4 days after parturition. The sugar was extracted six times with methyl alcohol; there remained 4 mg. of residue which was discarded. The six extracts were worked up separately and yielded six crystalline fractions with the following physical properties:

Fraction	M.P.	$[\alpha]_{5461}$ Initial	$[\alpha]_{5461}$ Final	C	H
1	138–140	117	60	39·71, 39·91	6·75, 6·73
2	146–147	125	68	39·96, 40·27	6·87, 6·79
3	139–140	120	60	39·87, 40·15	6·70, 6·85
4	140–145	125	63	39·94, 39·75	6·72, 6·69
5	148–150	124	64	40·17, 40·20	6·77, 6·73
6	148–150	126	67	39·95, 40·06	6·79, 6·72

The figures do not show a progressive rise in the melting points and specific rotations of successive fractions as would have been the case had

any appreciable amount of galactose been present in the sugar. The insuperable difficulty when a composite specimen of sugar from many different sources has to be examined is that only in a few cases may the sugar have been present, and before this has been worked up it has been diluted with much material that is valueless. The question must be left undecided. In the case of the examination of the blood sugar for lactose, the negative result is at first sight remarkable, in that the sugar was present in the urine and must therefore have been in the circulation; but it would indicate that this sugar on absorption from the mammary gland is only present momentarily in the blood, and there can be no threshold for it.

SUMMARY.

Neither lactose nor galactose has been detected in the blood sugar during lactation; there is no threshold for the former sugar.

I am grateful to Prof. D. Dougal for permission to obtain material from his cases at St Mary's Hospital. The expense of this work was in part defrayed by the Government Grant Committee of the Royal Society.

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612.745.3

A NEGATIVE PHASE IN THE HEAT PRODUCTION OF MUSCLE.

By W. HARTREE.

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In a previous paper [Hartree, 1932, p. 284] it was stated that negative delayed heat production had been frequently observed after the contraction of a frog's muscle at a low temperature in the absence of oxygen. It was suggested that the result might be due to a technical error, most likely to a small increase in the weight of the muscle between the times of taking the "live" and the "control" curves. Such an increase of weight, which might be due to a shortening and thickening of the portion of muscle on the thermopile, was shown to produce an effect similar to that observed.

It was possible to test this explanation of the negative delayed heat by making "control" curves on the living muscle mixed with curves recording the response to stimulation. Dr E. Bozler (personal communication) has employed a high-frequency oscillating current for this purpose. Prof. D. T. Harris was kind enough to supply Prof. A. V. Hill with the valve oscillator shown diagrammatically in Fig. 1, which gives a current of such high frequency that the muscle does not respond to it and yet not so high that the "skin effect" is appreciable. By means of this the living muscle can be sufficiently heated for control curves to be taken without any sign of stimulation. It was tested and confirmed that heating a muscle in this way has no harmful effect on its subsequent response.

The heating current was applied for about 0·05 sec. by allowing a revolving arm to open a short circuit key, which promptly fell back as soon as the arm had gone by. While the key was open the high-frequency current passed through the muscle between the usual electrodes.

By taking sets of control curves interspersed with the live curves it is evidently quite immaterial whether the weight of a muscle alters progressively during an experiment or not. It is interesting, however, that in every one of several experiments the control curves for 0·05 sec., heating, taken later on during an experiment, fell from their maxima

faster than did those taken earlier, which can only be explained by the muscle losing weight during the course of an experiment. The gas in the chamber is saturated, so this loss of weight cannot be due to evaporation: presumably a certain amount of liquid gradually drains out of or away from the tissue. The loss of weight is probably quite small, but it can hardly be estimated. Its effect would be in the opposite direction to that required to explain away the negative delayed heat as a technical error. That explanation, therefore, is invalid.

The effect of this gradual change in the control curve is to make the negative delayed heat previously described rather too small. A live curve is analysed by the correct control curve taken at about the same time, and also by a more quickly falling control curve taken much later. Using the same procedure as in the paper referred to, the analysis of the initial heat is hardly affected by the difference between the two control curves, but the calculated values of the effect at subsequent times of the initial heat are too small in the latter case, and these, subtracted from the live curve, give a set of negative numbers which are numerically too small. These numbers, however, are then analysed by a 4 sec. heating curve (built up from the corresponding control curve) which also falls rather too fast, with the result that the calculated negative heat is only about $\frac{1}{2}$ p.c. of the initial heat too small. Although this is a considerable fraction of the total negative heat (about − 5 p.c. of the initial heat in the case examined) it is of no great account, being at about the limit of observation.

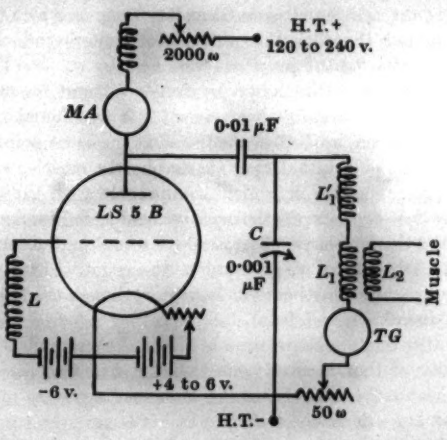

Fig. 1. Diagram of connections of high-frequency oscillator for heating without excitation. L and L'_1 are magnetically coupled (variable), and L_1 and L_2 are magnetically coupled (variable). The milliammeter (MA) measures the H.T. current and the thermogalvanometer (TG) measures the oscillator generator current. The frequency is about 100,000 oscillations per second.

Using the new method of making curves of control heating, a number of further experiments were carried out, all on pairs of sartorii of English *Rana temp.*, at 0° C., in nitrogen freed from oxygen by being passed over red-hot copper gauze. The low temperature is necessary since, at higher temperatures, the usual positive delayed heat previously described masks the negative.

The usual procedure was as follows. After about an hour (including dissection) in Ringer's fluid at room temperature the muscle, still in Ringer's fluid, was brought to 0° C., oxygen being bubbled for about 30 min. and then nitrogen for another 30 min. The Ringer's fluid was then blown out with nitrogen, and nitrogen still passed slowly through the chamber while one or two preliminary stimuli were given. Although desirable it was unfortunately impossible to get a reliable result for the first stimulus, since the initial heat for this was usually abnormal, probably due to the muscle not being in the same position on the thermopile before the first stimulus as it was afterwards.

Two thermopiles were used to see if the negative heat was in any way a function of the thermopile. In the one used most frequently, made by Mr A. C. Downing and referred to below as thermopile *H* [see Hartree, 1932, p. 274], the muscle pulled on the metal frame on which the thermopile was mounted; there was thus a danger of some thermo-elastic effect in the frame as the "cold" junctions were held by it. [See Feng, 1932, p. 458.] Any effect of this, however, cannot account for more than a small portion of the negative heat since (1) it was found that a very much greater tension, applied to the frame at the same point as that at which the muscle pulled, than that exerted by the muscle, was required to produce galvanometer deflections comparable with those on which the negative heat depends; (2) the negative heat lasted for a much longer time than the thermo-elastic effect; and (3) when the tension had much diminished at the end of an experiment, the negative heat was always numerically greater, sometimes much greater, though the thermo-elastic effect must then have been less.

In the other thermopile (referred to below as thermopile *T*) there was no possibility of thermo-elastic effect, but it had two disadvantages: (1) it lost heat rather quickly, so the later readings were rather small; (2) the chamber was made of ebonite and it is strongly suspected that this, or defects in the tube leading to the chamber, may have supplied the muscle with a small amount of oxygen (possibly dissolved in the ebonite) because it was distinctly observable that the response when using this thermopile held up much longer than when using the former

one. This may well account for the result which is admitted that the second thermopile gave appreciably less negative heat than the first, as the numbers given subsequently will show.

Results.

Care was taken in the calculation of the negative heat not to include any results if these contained early negative heat such as would occur if there were any inactivity in the outer layers of the muscle far from the thermopile [see Hartree, 1932, p. 284]. Such cases are easily detected, as the apparent negative heat then either cuts off the end of the relaxation heat or appears as a quickly falling negative rate only lasting for a second or two. If, on the other hand, there is a distinct break (*i.e.* no observable heat of any kind) for a second or two after relaxation, and if then the negative heat starts with an increasing rate, it is considered as genuine, even though this occurs before 4 sec. after a short stimulus. There was, however, in many cases a very good "wash-out" of the numbers in the analysis between the end of relaxation and 4 sec. (for stimuli not longer than 1 sec.). Even then, however, it is remarkable that the analysis of the later negative heat carried out in steps of 4 sec. and starting at time 4 sec. from the beginning of the stimulus, nearly always showed a maximum rate of negative heat between 4 and 8 sec. For this reason the initial analysis was carried on in a few cases for 8 sec. and a typical result is shown in Fig. 2, where, although there was no observable negative heat before 4 sec., its maximum rate was at about 6 sec.

The result is not greatly different for any time of stimulus from 0·1 sec. up to 1 sec. so long as the negative heat rate is expressed as a fraction of the initial heat; the amounts, however, are very different in different experiments, and in any one experiment the negative heat always increased for subsequent stimuli, being frequently much greater for the second record than for the first, the latter being usually taken after one or two preliminary stimuli of very short duration. This may perhaps account for the diminution of the positive delayed heat for successive stimuli observed at higher temperatures.

For longer stimuli, 4 to 5 sec., the total negative heat was always less than might have been expected (according to the usual increase just mentioned) and it ended earlier. This is no doubt due to the earlier occurrence of the positive delayed heat in such cases (as has been shown at higher temperatures [see Hartree, 1932, Fig. 2, p. 278]. The onset of the positive delayed heat masks the end of the negative heat. After a 10 sec. stimulus there was no observable negative heat, although for a

1 sec. stimulus taken before and for another after, there was large negative heat (− 5 and − 7 p.c.) going on for a long time (40 and 50 sec.). Such cases, therefore, were discarded; and the totals subsequently mentioned refer to stimuli of 1 sec. or less.

For an early stimulus, usually after one or two preliminary stimuli, the total negative heat was from 0 to − 2 p.c., in all cases for thermopile

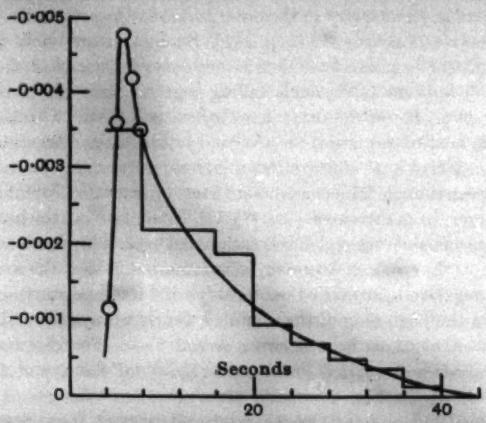

Fig. 2. Negative heat rate subsequent to a stimulus of 1 sec. at 0° C. (taken after several others). There was no observable heat of any kind between 2 and 4 sec. The stepped line shows the result of the usual analysis of the later heat by 4 sec. intervals. Circles show the result of the initial analysis being carried to 8 sec. by steps of 0·8 sec. The actual form of the curve, though smooth and similar to that in other cases, is hardly to be relied upon as it depends on very small differences. The end of the curve is not asymptotic with the base as a small positive heat rate supervened. The vertical scale is in terms of the initial heat per sec. and the total negative heat is in this case − 5 p.c. of the initial heat.

T and in several cases also for thermopile H: for the latter, however, it was much more (up to − 6 p.c.) in a few cases.

After several stimuli and a considerable time thermopile T gave a total of − 2 p.c. in several cases, going up to − 6 p.c. in a few cases (average − 3½ p.c. for seven cases). Thermopile H, on the other hand, never gave numerically less than − 4 p.c., and in four cases the total was up to − 9 p.c. (average − 7 p.c. for eleven cases). The averages are of no particular value beyond showing the different results arrived at by the

different thermopiles, since there is no indication that the maximum negative total was reached in any case—the positive delayed heat probably always interfered with it to some degree. If, as explained above, readings of thermopile *T* are open to some doubt, the maximum negative heat is probably about the same (8 p.c.) as that found for the positive delayed heat in the absence of oxygen for fresh muscle. [See Hartree, 1932, p. 278.]

As regards the distribution of the negative heat this appears to depend little on the length of the stimulus. After a long stimulus of 4 sec. there is quite a good "break" or "wash-out" between relaxation and 8 sec., so that the negative heat does not start with an exceptionally large early rate. The maximum negative rate in fact depends only on the total negative heat, being from $-0{\cdot}0007$ to $-0{\cdot}001 \times$ (initial heat per sec.) for each 1 p.c. of negative heat, and this occurs between 4 and 8 sec. after the end of the stimulus (including stimuli up to 4 sec.).

The time to completion of the negative heat is shown approximately by the following (rough average of about 35 observations):

Total negative heat: p.c. of initial	1	2	3	4	5	6	8
Time of completion: seconds	15	25	30	35	40	45	50

In a few cases the greater totals required 60 sec. for completion. Of course the time of the end of the slowly descending curve is very indefinite, and this would be considerably affected by traces of oxygen and by differences in the occurrence of the positive delayed heat after different stimuli, but the average of a large number of observations will give a good enough general idea.

It should be noted that the maximum tension for successive contractions fell from the start, and in the later records (after 2 or 3 hours) it was sometimes not more than half the original value; the initial heat also fell for the later records, though there was sometimes a distinct rise for the earlier ones, even though the maximum tension was falling. In every case the percentage fall of tension was greater, and in many cases very much greater, than that of the initial heat[1]. It seems, therefore, that the state of the muscle, especially when the negative heat was large, was very different from that of fresh muscle: it must be understood, however, that the muscles were not irreversibly changed by their treatment, as in all but one of about 20 cases after several hours in nitrogen they were perfectly restored after being left an hour or two in oxygenated Ringer's fluid, the final response being often slightly larger than it was initially.

[1] As has been observed before [Hartree 1931, p. 13].

As for the cause of the negative heat observed little can be said. It is natural to associate it with the earlier stages of a known endothermic reaction, namely the restoration of phosphagen which takes place in muscles deprived of oxygen at the expense of energy liberated by lactic acid formation. It will be of interest to find whether at 0° C. the time relations of phosphagen resynthesis are at all similar to those of the negative delayed heat. If the positive free energy of lactic acid formation were greater than the negative free energy of phosphagen resynthesis, the former might in principle drive the latter, even though the total energy of the combined reaction were negative. In part at least of the cycle the endothermic process has at 0° C. outstripped the exothermic one.

Summary.

1. By means of a high frequency oscillating current (about 100,000 cycles per sec.) a muscle may be warmed without being excited; this supplies a means, in myothermic experiments, by which "controls" can be made at intervals during a series of observations on living muscles.

2. This avoids the possibility of errors due to a progressive change in shape, weight or position of a muscle on a thermopile.

3. In a frog's muscle at 0° C. after a short tetanus, there is a negative delayed heat production which is usually very small for early stimuli and this increases, for later stimuli, up to about 8 p.c. of the initial heat. This heat is complete in 30 to 60 sec. when it is followed by the usual positive delayed heat which, at higher temperatures, comes on so quickly as to mask it completely.

4. This negative delayed heat may be associated with the endothermic restoration of phosphagen, but further experiments on the time relations of that process are necessary before the connection is certain.

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THE HEAT PRODUCTION OF CRUSTACEAN NERVE.

By MARY BERESINA[1] and T. P. FENG[2].

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Hill in 1929 investigated the heat production of crab's nerve. The total heat per sec. of maximal stimulation was estimated to be at least $2{\cdot}5 \times 10^{-3}$ cal./g. of moist nerve, and a clear division of this heat into an initial and a recovery phase was brought out by analysis, the former representing about 2½ p.c. of the total heat. In his work, however, photographic recording was not used, and analysis was only carried out in 5 or 6 sec. units. It was expected that finer details of the course of the heat production would be revealed by the use of photographic recording and by analysis in shorter units.

A number of points, moreover, in the energetics of crab's nerve remained to be examined: (1) the "optimal" stimulus; (2) the effect of frequency of stimulus; (3) the heat per single isolated impulse; (4) whether the course of the heat production in nitrogen is different from that in oxygen; (5) whether the natural state of steady activity revealed in the steady contraction of its attached muscle can be artificially realized by a sufficiently slow rate of stimulation; and (6) its resting heat production. The methods and technique developed recently by Prof. A. V. Hill [1932] for an investigation of the heat production of frog's nerve supplied the means for carrying out the programme described.

Procedure.

Three thermopiles were employed: for the determination of resting heat an old silver-plated differential thermopile [see Beresina, 1932] and a new mica-faced silver-plated one: for heat production due to stimulation the new soldered thermopile referred to by Hill [1932, p. 112] as not ready in time for his experiments. A Kipp Zc galvanometer (period 5·5 sec.) was used in most experiments both for resting and stimulation heat production, occasionally a Zd (period 3·8 sec.) for the latter to gain quickness. Resting heat production was studied in a gas-heated water thermostat at 20·7° C., while a Dewar flask was employed for stimulation experiments. Stimulation was always by repetitive condenser charge and discharge with the aid of a commutator [Hill, 1932].

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The limb nerves of *Maia* were employed throughout. Six to eight nerves were required, and on a warm day nerves dissected first were kept in a sea-water bath at about 12° C. Usually two persons did the dissection together, and mounting of the nerves on the thermopile was begun by one person as the last nerve was being dissected by the other. After some practice the total time from the beginning of dissection to the placing of the thermopile with mounted nerves in the Dewar flask was about 30 to 50 min. Considering the rather rapid deterioration of crab's nerve, especially at higher temperatures, it is important that dissection should be quickly done. The nerves were then soaked in oxygenated sea water in the thermopile chamber for about 20 min., which suffices for equalization of temperature, and apparently also for removing the depressed excitability due to dissection and handling. The sea water was then sucked out and the chamber filled with moist oxygen. Usually in another quarter of an hour the conditions would be sufficiently steady to begin observations.

Photographic records were always taken in experiments in which analysis of heat production was intended.

Optimal stimulus.

For the theory of the optimal stimulus and its importance in connection with the question of "heat leak" in neurothermic investigations we refer to Hill [1932]. In the study of crab's nerve heat the use of stimuli with minimal energy is necessitated by the facts (i) that crustacean nerve is easily injured by strong stimuli, and (ii) that, if stimuli of improper characteristics are used (*e.g.* break induction shocks), the energy in them may need to be too great.

In Fig. 1 are three curves from three separate experiments, relating the size of response to the electrical capacity of stimulus at constant energy. The curves do not possess such perfect symmetry as those for frog's nerve given by Hill [1932], but the existence of an optimal capacity is shown beyond doubt. In *A* and *B* the resistance of the stimulating circuit was 1560 ohms, in *C* 840 ohms. Reading off the capacity (*F*) corresponding to maxima from the curves, the optimal values of *RF* in the three experiments are 1150, 1290 and 940 respectively, averaging (say) 1200. In subsequent experiments the stimulus was always made to possess an *RF* in the neighbourhood of this value. It may be noted that the optimal *RF* for crab's nerve is about six times that for frog's nerve [Hill, 1932].

In cases, such as this and the next, in which no analysis was necessary, the size of response was determined as follows. The ordinates at the fourth, fifth and sixth seconds of the photographic record of each response were measured and their sum taken. This sum was used as the measure of the response. It was assumed that the curves of heat production due to stimuli of a given duration are all of the same shape, differing only in size, so that the corresponding points of all curves are comparable. The reason for choosing the fourth, fifth

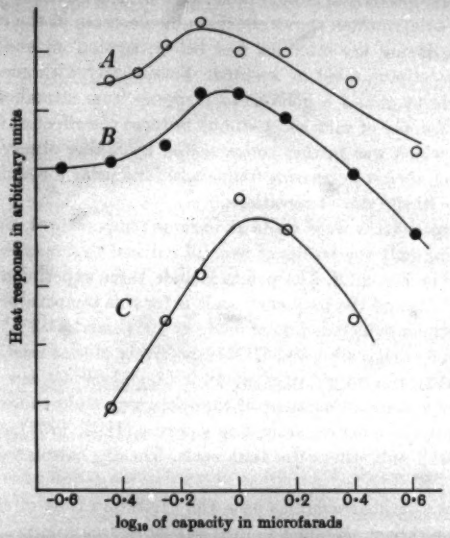

Fig. 1. Three determinations of optimal stimulus at about 17° C. Curves *A* and *B* were obtained with 1 sec. stimuli at about 90 per sec., *C* with 2 sec. stimuli at the same frequency. In each determination the energy of the stimulus (proportional to FV^2) was kept constant. The resistance (R) in series with the stimulating condenser (F) was 1560 ohms for *A* and *B* and 840 ohms for *C*. The product of the capacity in microfarads corresponding to the maximum of the curves and the resistance in series with it, or in short the optimal RF, was 1150, 1290 and 940 for *A*, *B* and *C* respectively, averaging about 1200.

and sixth seconds readings is merely that they are the earliest points on or near the horizontal limb of the curve (see Fig. 3, *A*) where measurement is easier for two reasons: (1) the portion of curve concerned is nearly flat so that a small displacement in time will introduce no error; (2) its proximity to the beginning of the curve reduces error due to any inaccuracy of the base line. The assumption stated may be inexact for long stimuli since varying degrees of failure during the stimulus would occur for different frequencies; for 1 or 2 sec. stimuli, however, it must be very nearly true.

Frequency of stimulation.

There are serious difficulties in determining the effect of frequency of stimulation upon the heat production in crab's nerve. Its rapid spontaneous deterioration and its ready fatigability tend to make successive observations not strictly comparable. Even in a stimulus as short as 4 sec. crab's nerve often shows considerable decrease in the rate of heat production during the stimulus (see below, section on analysis). Obviously long stimuli must be avoided. Fortunately with good nerves it was possible to obtain a good-sized response from stimuli as short as 1 or 2 sec. The use of such short stimuli reduces the effect of fatigue to a minimum, which was further compensated by taking observations first in increasing, then in decreasing frequencies, and using for each frequency the average of the two observations.

Nine experiments were made at various temperatures, but to avoid overcrowding only the results of five, all reduced to a maximum of 300, are plotted in Fig. 2, *A*. The points include three experiments made at about 16·9° C., and the frequency scale is for this temperature. The two other experiments included were made at 10° C. and 11·3° C., and their real frequency scales are $\frac{1}{2}$ and 1/1·8 respectively of that used in the plot. The fact that results obtained at 16·9° C. and 10° C. can be plotted together by a mere adjustment of the frequency scale shows that temperature, in crab's nerves as in frog's nerves [Hill, 1932] and muscles [Feng, 1931], only alters the time scale. The Q_{10} calculated from this relation is 2·9.

From the optimal stimulus data the excitation time of crab's nerve appears to be about six times as long as that of frog's nerve. This would lead one to expect that the maximum frequency of stimulation also for crab's nerve would be very much lower than that for frog's nerve. Comparing, however, Fig. 2, *A* of the present paper for crab's nerve at about 16·9° C. with Fig. 5 of Hill's [1932] for frog's nerve at 21·3° C. it will be noted that the difference in the frequency scale falls very short of expectation. The maximum frequency for crab's nerve at 16·9° C. may be taken to be about 200 per sec.; at 21·3° C., on the basis of a $Q_{10} = 3$, it would be about 320 per sec. This does not differ much from the maximum frequency of about 400 per sec. for frog's nerve at 21·3° C. (The value 400 per sec. is used for comparison, since the increase of heat response caused by frequencies higher than this is very slight. See Hill, 1932, Fig. 5.)

The large difference in optimal stimulus and the small discrepancy

in maximum frequency between frog's and crab's nerve need not, however, be contradictory. The optimal stimulus depends primarily upon the "excitation time"—indeed it can be derived from the strength-duration curve of nerve excitation, except for the complication that the optimal stimulus of the present work is determined for tetanic stimuli of 1 to 2 sec. duration, while the strength-duration curves are generally found by

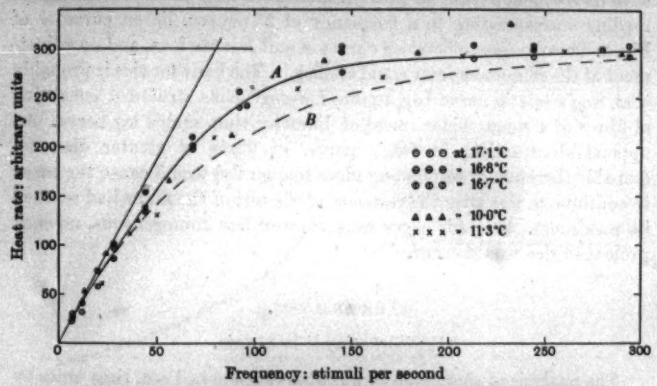

Fig. 2. The relation between heat production of nerve and frequency of stimulation. *A* is the mean curve for crab's nerve drawn through the points of (*a*) three experiments made at about 16·9° C., and (*b*) two at 10° C. and 11·3° C., the frequency scales for the latter having been multiplied by 2 and 1·8 respectively in order to plot them together with the three experiments at the higher temperature. The maxima of the curves of individual experiments have all been arbitrarily reduced to 300. Observations made at widely different temperatures are seen to lie sensibly on a single curve after an adjustment of time scale. *B* is Hill's [1932, Fig. 5] mean frequency curve for frog's nerve reproduced after reduction to the same maximum of 300 with its time scale for 21·3° C. multiplied by 0·72 so as to make the reading for frequency 25 per sec. lie on curve *A*. The divergence between *A* and *B* is definite, showing that the results from frog's and crab's nerves cannot be made to coincide, as those from the same nerve at different temperatures can. The tangent to curve *A* at the origin intersects the level of maximum heat production at a point whose horizontal coordinate is 80 per sec.

single minimal shocks. The frequency effect would be primarily related to the refractory period, or the curve for recovery of excitability after the transmission of an impulse. While on general grounds in similar structures quick excitation time is expected to be associated with short refractory period, they may not run parallel in structures so different as medullated and unmedullated nerve.

Another comparison is possible between the frequency curves of frog's and crab's nerves. If the nerves of frog and crab differed only in quickness, a proper adjustment of their frequency scales should bring their results to lie on a single curve, as is the case with the same type of nerve at different temperatures. Curve *B* in Fig. 2 is the mean frequency curve given by Hill [1932, Fig. 5] reduced to a maximum = 300, and with its frequency scale for 21·3° C. multiplied by 0·72 so as to make the reading corresponding to a frequency of 25 per sec. lie on curve *A* of Fig. 2. The divergence between curves *A* and *B* is obvious, and no adjustment of the frequency scale could abolish it. The basis for this is probably that frog's sciatic nerve has a more heterogeneous structure, consisting of fibres of a much wider range of diameter than crab's leg nerve. An appreciable fraction, in frog's nerve, of fibres of greater diameter (capable therefore of responding more frequently) would cause the curve to continue to rise after the response of the rest of the fibres had reached its maximum. If crab's nerve were more or less homogeneous, no such prolonged rise would occur.

The analysis.

(1) *Short stimuli in oxygen.*

The analysis of short stimuli was always made in 1 sec. time units by means of 1 sec. heating controls. For the latter the mean of three or four curves was generally used. The number of live curves used for analysis varied for different durations of stimulus and in different experiments. For 1 sec. stimulus the sum of three to six curves was generally employed; for 4 sec. or longer stimuli, one to three curves. Compared with the case of frog's nerve such numbers seem small. They should, however, give accuracy enough, considering the size and smoothness of the records obtained (see Fig. 3), and the great susceptibility to fatigue and the rapid spontaneous deterioration of crab's nerve render it impossible to make a large number of records, as is feasible with frog's nerve. Generally, stimuli of durations from 1 to 4 sec. were given at intervals of 8 to 12 min., and four or five similar records of 4 sec. stimulus were about the most that could be secured.

Fig. 3 shows typical records of heat production due to short stimuli: *A*, *B*, *C* are 1 sec., 4 sec. and 8 sec. stimuli respectively, while *D* is the 1 sec. heating control by means of which the stimulus curves are to be analysed. They are all from one experiment made at 15·5° C. Fig. 3, *E* is another 4 sec. stimulus from an experiment at 20·5° C.

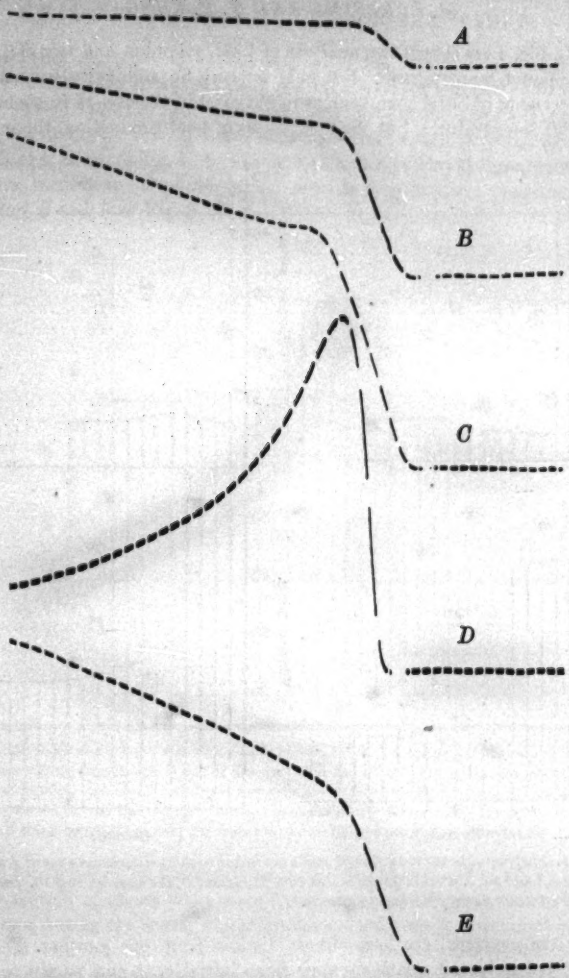

Fig. 3. Photographic records of heat production. *A*, *B* and *C* are 1, 2 and 4 sec. stimuli, 92 per sec. at 15·5° C. *D* is a 1 sec. heating control due to 380×10^{-6} cal./g. This is intended also to convey some idea as to the sensitivity of our galvanometer-thermopile system. *E* is another 4 sec. stimulus at 20·5° C. Compare *C* and *E*. The higher rate of recovery heat production at the higher temperature is evident from the records without analysis. Time marks every second.

In Fig. 4 are shown two analyses of 1 sec. stimulus, and two of 4 sec. at different temperatures. Not only is there no ambiguity concerning the presence of intial heat, such as might exist in the case of frog's nerve at high temperatures, but the two phases of heat production, the initial

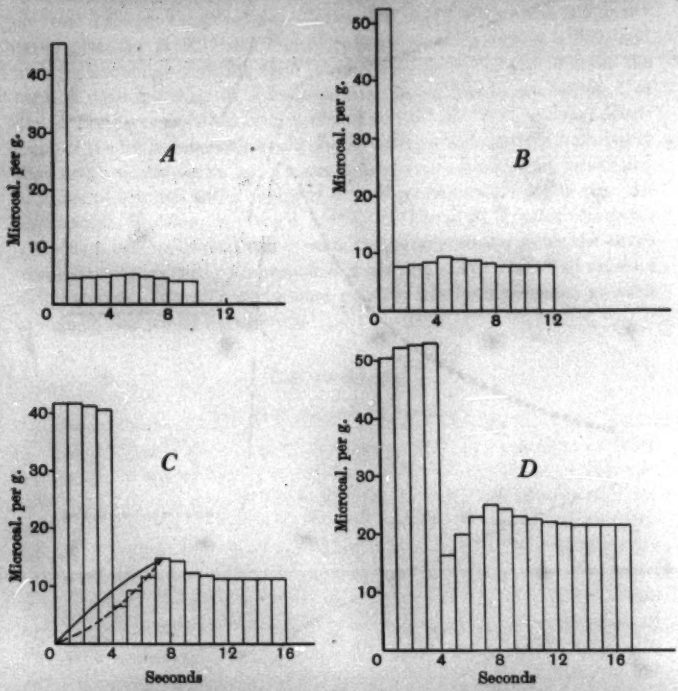

Fig. 4. Analyses of heat production of crab's nerve due to short stimuli in oxygen: *A* and *C* are 1 and 4 sec. stimuli, 92 per sec. at 15·5° C., *B* and *D*, the same at 20·5° C. See text for further description and comments.

and the recovery, are here almost distinct from one another. This is partly due to the relatively very large initial heat and partly to the fact that the maximum rate of recovery is not reached until some seconds after the stimulus is over, so that at the end of the stimulus there is a very abrupt and striking fall of heat rate followed by a "hump"

representing the recovery maximum. After the "hump" the recovery heat rate continues at a practically constant level for a period of at least 30 to 40 sec. That the rate of recovery heat production after a stimulus is higher at higher temperatures is evident from comparing analyses *A* and *B*, or *C* and *D* of Fig. 4. But to show this, analysis is hardly necessary: a mere inspection of photographic records is sufficient; compare for instance *B* and *E* of Fig. 3.

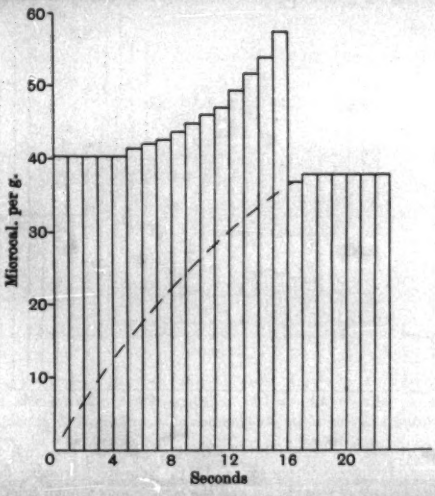

Fig. 5. Analysis of a 16 sec. stimulus, 92 per sec. at 18° C. Its somewhat unusual appearance during stimulation is due to the fact that the initial heat rate is decreasing, at first rapidly, then slowly, while the recovery heat rate is steadily increasing. The blocks above the dotted line may be taken as approximately representing the initial heats, and those below it the recovery heats in the successive seconds of stimulation.

In frog's nerve, at temperatures in the neighbourhood of 20° C. and using stimuli of about 200 per sec., there is always a great increase of heat rate during the course of a stimulus, indeed even for stimuli as long as 4 min. or more. This is, of course, largely due to the quickly increasing recovery heat summed together with the initial heat, but also to the relative constancy of the latter, as an inspection of Hill's analyses [1932] will show. In the case of crab's nerves, even in a stimulus as short as 4 sec., when the frequency of stimulus is over 60 per sec., there is either

only a slight rise in the heat rate or, as is more commonly the case, an actual fall. From the frequency curve it can be seen that a frequency of 60 to 90 per sec. is still a long way from the maximum for crab's nerve, and is relatively not higher but lower than 200 per sec. for frog's nerve at similar temperatures. Thus crab's nerve stimulated even at moderate frequencies may show pronounced "fatigue" in as short a time as 4 sec.

Fig. 5 shows the analysis of a 16 sec. stimulus at 92 per sec. at 18° C. The shape of the analysis appears unusual but easily intelligible once it

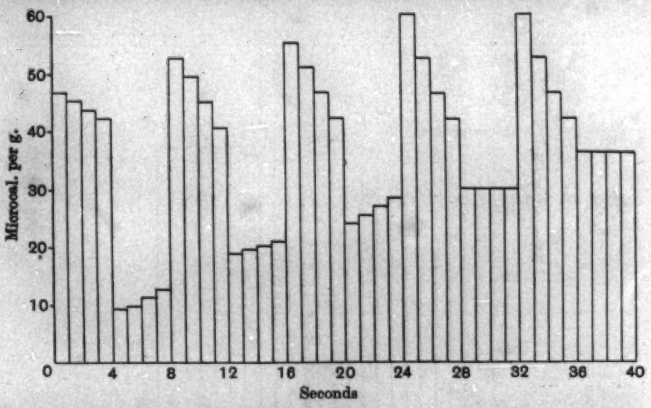

Fig. 6. Analysis of heat from intermittent stimulus, 4 sec. on, 4 sec. off, taken with Zd galvanometer. Frequency 92 per sec., at 17° C. Note the increasing degree of failure in successive periods of stimulation and the conspicuous though brief recovery after each rest interval.

is remembered that during the course of the stimulus the recovery heat rate is continuously increasing while the initial heat rate is decreasing, rapidly at first, then slowly. Clearly the sum of the recovery heat and the initial heat changing respectively in the manner described will result in the peculiar course of heat production as shown. In Fig. 5 the blocks below the dotted line may be taken as approximately representing the recovery heat rates in successive seconds of the stimulus, those above it, the corresponding initial heat rates.

Concerning the progressive failure of the initial heat rate, the following type of experiment is of interest. Instead of giving a 16 sec. or longer stimulus continuously, it may be split into 4 sec. periods, separated by

4 sec. gaps. The result of such an experiment is given in Fig. 6. We see (*a*) the first 1 sec. block of each 4 sec. stimulus is always higher than the last block of the preceding 4 sec. stimulus, showing that a rest of even 4 sec. can bring about considerable recovery; (*b*) while in all five periods of stimulation there is distinct failure of initial heat rate, the failure is more rapid in the later periods, showing the accumulated effect of previous stimulation, the recovery achieved in 4 sec. rest being very incomplete.

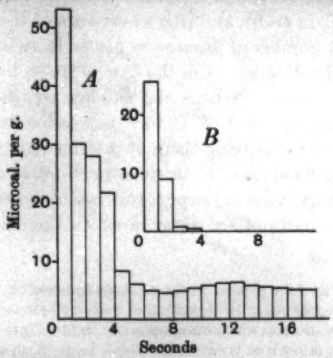

Fig. 7. Showing the very pronounced failure of crab's nerves at lower temperatures when stimulated at relatively high frequency. *A*, 4 sec. stimulus, 92 per sec. at 10·5° C. Note the striking decline of initial heat during the stimulus and also that the heat rate at the end of the stimulus did not fall suddenly to a low level rising again immediately afterwards, but dropped to a level which further decreased for 4 sec. before showing the usual rise. Compare this latter feature with Fig. 9 and see text for its discussion. *B*, 4 sec. stimulus, 60 per sec. at 0° C. The failure is striking. The last 2 sec. stimulation was hardly effective at all, and after the stimulus there was no significant recovery heat production up to the end of analysis. Scale same as *A*.

At lower temperatures the failure of crab's nerve during stimulation is very much more pronounced. In Fig. 7 are *A*, the analysis of a 4 sec. stimulus at 92 per sec. at about 10·5° C., and *B*, that of a 4 sec. stimulus at 60 per sec. at 0° C. In *A* the initial heat drops sharply after the first second and continues to fall in the course of the 4 sec. stimulation. The recovery heat at this temperature, as is to be expected, is produced at a relatively lower rate, reaching a maximum also somewhat later. The later maximum, however, is also due to another cause in this particular case, as will be explained later on. At 0° C. the failure of crab's nerve is striking. The record of which the analysis is shown in Fig. 7 *B* is very

small, but smooth, and as far as we could make out, Fig. 7, *B* is the approximate solution. After 2 sec. the heat production has become practically nothing and continues to be so until at least the twelfth second. The recovery rate is so slow that the nerve seems not in a position to respond to many stimuli in succession. That no irreversible damage was caused by the cold was found by warming the nerve up again to 17° C. when it gave good normal responses. Hill [1932] found that frog's nerves, too, will not work well if suddenly transferred to 0° C. Whether the failure at 0° C. in crab's and frog's nerve are of the same nature it is difficult to say. A number of *Maia* were placed in an ice box in the hope of producing acclimatization as in the case of frogs, but they all died in the box after one day. Perhaps the sudden transference from their normal environment of about 13° C. to 0° C. was too drastic a procedure. Facilities, however, for keeping them at intermediate temperatures and gradually working down to 0° C. in steps were not at our disposal. One possible contributory cause of the poor survival of the crabs in the ice box might be that the basin of sea water in which the crabs were kept was not aerated.

The analyses in Fig. 4 we present as the most typical results. In Fig. 7, *A*, coming before the "hump" representing the maximum of recovery heat rate, there is a small tail piece at the end of the stimulus which cannot possibly be fitted into the course of recovery heat production if the latter is to preserve any simple form. Such a tail piece occurred in about 30 p.c. of our records and appeared to be usually, but not always, related to a very pronounced failure of heat production during the course of the stimulus when the latter was longer than 1 sec. On the other hand it occurred relatively most frequently in analyses of 1 sec. stimuli, three out of seven cases, while in analyses of 4 sec. stimuli it occurred only in four out of sixteen. Further, in analyses in which it occurred the "hump" came later. Fig. 8 shows the analysis of a 2 sec. stimulus with conspicuous tail piece and delayed recovery maximum.

It is well known that an isolated crab's leg is capable of tonic contraction, and Barnes [1930] has reported persistent discharges in the motor nerve of *Cancer pagurus* as a result of cutting, pinching, ligaturing or traction. It is conceivable then that if the stimulus used in some of our experiments had been too strong, some injury might be produced at the site of stimulation, so setting up a short period of persistent discharges after the stimulus is over. This, if true, would account for the tail piece under consideration. Unfortunately the suggestion did not stand experimental test. In one experiment, after a number of records with the usual stimulus, the strength of stimulus was increased fifteen times. The result turned out to be diametrically the opposite of that expected: the analysis of some of the records taken with the usual stimulus showed the tail piece, while that of records obtained with the unusually strong stimulus did not. Persistent discharges aside, there appears no other way of understanding the tail piece as a physiological phenomenon. To compare it with the delayed anaerobic heat production in muscle seems hardly justifiable.

It remained therefore to be seen whether the tail piece could be regarded as an error. In muscle it has been confirmed [Blaschko, 1930] that if a piece of filter paper be interposed between the thermopile surface and the muscle there will appear an artificial

delayed heat production. Now if, of the six to eight nerves used, the centrally placed ones, viz. those directly lying on the hot junctions, were inactive during a short stimulus, the effect would be very similar to having a piece of filter paper in between the thermopile and the nerves, and delayed heat production or tail piece would be obtained.

That such is actually the case was shown by the following experiment. Six nerves were carefully dissected and three mounted on each side of the thermopile surface. Four records of 2 sec. stimulus and three control curves having been obtained, the middle nerve on each side was partly crushed below the stimulating electrodes. Records of 2 sec. stimuli and controls were again taken with everything precisely the same as before. Fig. 9 gives the analyses of the records before and after damaging the central nerves. The result is clear-cut:

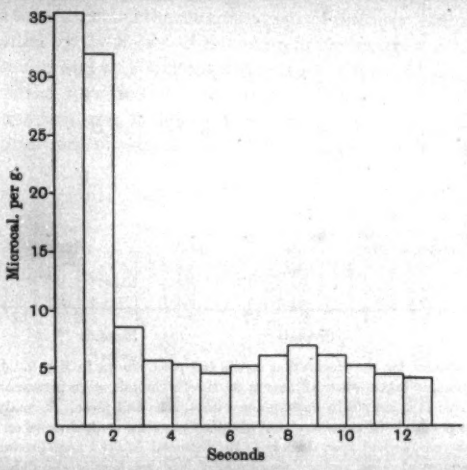

Fig. 8. Analysis of heat from 2 sec. stimulus, 90 per sec. at 15·7° C. showing the extra falling bit of heat production following the end of stimulus (or tail piece) and delayed recovery heat maximum.

analyses such as that of Fig. 8 can be produced artificially. The reproduction goes down even to details: there is the pronounced failure before the tail piece and the hump representing the recovery maximum is also delayed.

In the case of frog's nerves the chance of a large part or the whole of a nerve being inactive, assuming nerves from healthy animals to begin with, is small, frog's nerves being so resistant. With crab's nerves, however, the chance is larger. In the first place some fibres must have been cut or torn if a nerve was cleanly dissected, or else some tissue tags must have remained attached to it when its isolation was not so cleanly done; in either case a certain amount of inactive matter would be present in the preparation. In the second place, of the nerves placed on the thermopile some may have deteriorated more rapidly than others, and at the time of stimulating they may have been only partially active: such nerves may happen to have been centrally placed. Thirdly, since an over-maximal stimulus

is not safe [Furusawa, 1929], the approximately maximal stimulus employed may not have been adequate for some of the nerves, and these again may have been centrally placed. The experiment with the unusually strong stimulus related above, while failing to achieve its original purpose, is of interest in this connection. In that experiment the usual stimulus was most probably too weak for a good many fibres, particularly for those of the nerves occupying a central position, so producing a response whose analysis showed the tail piece, while the unusual stimulus, strong enough to stimulate all the nerves, also got rid of this appendage.

It may be objected to this explanation of the effect discussed that the converse case of apparent negative heat appearing at the end of the stimulus should also sometimes occur:

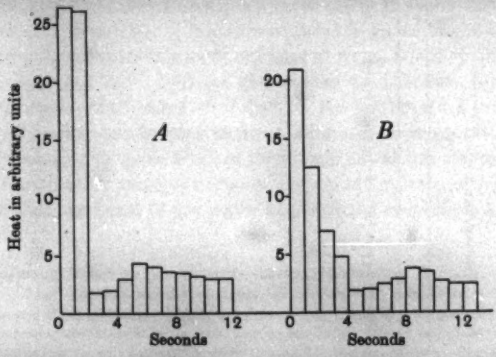

Fig. 9. Experiment for the elucidation of the tail piece shown in Fig. 9. *A*, analysis of 2 sec. stimulus taken when all nerves on the thermopile were presumably excitable and active; it is simple in appearance without the tail piece. *B*, analysis of 2 sec. stimulus precisely the same as *A* but obtained after the central nerve on each surface of the thermopile had been damaged; the artificial delayed heat production (or tail piece) is most obvious, and the maximum of recovery heat is also delayed. There is besides the very pronounced failure during the stimulus. Thus, by merely damaging the nerves occupying the centre position on each thermopile surface, it is possible to reproduce analyses like that in Fig. 9.

the central nerve, the one on the thermo-junctions, might be more and not less active than the average of the rest. As a matter of fact such cases do appear but not very obviously or often. Fig. 4, *C* may serve as an instance. There the course of the recovery heat production found by analysis follows the dotted line: the true course is most probably more like the full line; negative heat would account for the discrepancy.

The reason for the relative infrequency and the smallness, when it appears, of the converse effect is probably as follows. In determining the form of the record, the two nerves lying actually on the warm junctions of the thermopile exercise a more immediate influence than the rest. Their activity, like that of any other single nerve, may vary from zero on the one side up to a certain maximum on the other. Since, however, an experiment is always discontinued when the nerves are showing obvious signs of failure, the average of the six or eight nerves used must always be rather high. Representing maximum

excitability by 100, that of the pair of nerves on the junctions may vary from 0 to 100, while the average of all the nerves would never be much less than 70. It is just as likely that the central nerves on the junctions should be above or below the rest in respect of their excitability, but the amount by which they can be above is much less than the amount by which they can be below, and it is only those cases in which the difference between the central nerves and the rest is large enough which will show any obvious sign of the effect under discussion. It is natural, therefore, that the converse case of apparent negative heat following the stimulus should be relatively infrequent and the amount, when it occurs, be small.

(2) *The maximum recovery rate for a short stimulus.*

As was to be expected the maximum rate of recovery heat production, as represented by the height of the hump in the analysis, is higher for a longer stimulus and at a higher temperature. Expressed, however, as a fraction of the total initial heat, while preserving the difference for different temperatures, it does not seem to be consistently different for different durations of stimulus, at least to 12 sec. Table I summarizes

TABLE I.

No. of analysis	Temperature °C.	Duration of stimulus sec.	Maximum recovery heat rate, p.c. of total initial heat
1	10·5	4	6·7
2	10·5	4	5·0
3	11·0	12	5·3
4	10·5	4	6·0
5	10·5	2	5·0
Average	10·6		5·6
6	17·2	4	10·7
7	15·8	1	11·4
8	16·8	1	14·2
9	16·4	1	14·8
10	15·8	1	14·2
11	16·3	1	13·5
12	15·9	4	9·8
13	16·3	4	11·8
14	15·7	6	8·0
15	15·8	8	8·1
16	16·6	4	12·0
17	16·3	4	12·0
18	16·9	2	12·0
19	17·0	2	11·0
20	17·0	2	14·0
21	15·5	2	11·5
22	16·3	2	11·7
23	16·6	2	14·3
24	16·6	2	11·4
Average	16·4		12·2
25	20·5	4	14·0
26	20·0	4	15·7
27	19·3	4	16·0
28	20·5	1	18·6
Average	20·1		16·1

the analyses with respect to this point. The average maximum recovery heat production per second, as a percentage of the total initial heat, had the following values:

At about 10·5° C.	...	...	5·6 p.c.
At about 16·5° C.	...	...	12·2 p.c.
At about 20° C.	...	...	16·1 p.c.

These values will be used later in the discussion of the heat production of crab's nerve in nitrogen.

Hill [1929] states that the maximum rate of recovery heat production occurs almost immediately after the stimulus, and is about 6½ p.c. of the initial heat per sec. at 15° C. The first statement depends upon the fact that he made his analysis in 5 or 6 sec. units and so missed the hump. The second does not agree well with our result; this, however, is partly accounted for by our taking the hump as the recovery maximum, which is of course higher than the average recovery heat rate over 5 or 6 sec. after the stimulus, as used by Hill. Moreover his "initial" heat, owing to the long interval of analysis, contained some recovery heat, which would tend to reduce the fraction calculated.

(3) *The magnitude of the initial heat.*

(*a*) *Initial heat per sec. of maximum stimulation per g. of moist nerve.* The size of initial heat is naturally different for stimuli of different frequencies. It will serve no good purpose to record here all the values obtained at different frequencies under various conditions. Suffice it to collect the values of initial heat for fresh nerve at temperatures around 16·5° C. and stimulated at about 90 per sec.

TABLE II.

Exp. no.	1	2	3	4	5	6
Initial heat in microcal./g. × sec.	40·9	41·6	37·0	39·3	61·5	45·3
Exp. no.	7	8	9	10	11	Average
Initial heat in microcal./g. × sec.	65·5	38·1	53	37	43	45·7

About half the above results are from 1 sec. stimulus, the others from 2 or 4 sec. stimulus, in which case the heat rate during the first second is alone considered. We did not test in each case whether the stimulus was maximal or not. In cases where this was tested, increasing the voltage of the stimulus beyond that usually employed produced no significant increase of heat production. We feel safe to state in general that the strength of stimulus we employed could not have produced a response

smaller than 80 p.c. of the maximum. There is an additional factor, however, which must have tended to make the observed value of initial heat per g. low, and that is the probable presence of cut fibres in experiments in which dissection was very clean, or of tissue tags in those in which dissection was not so thorough.

(*b*) *The initial heat per single impulse.* From the intersection of the tangent to the frequency curve (Fig. 2, *A*) at zero time and the horizontal line representing the level of heat production corresponding to maximum frequency, it may be inferred that eighty isolated impulses would produce the same amount of heat as 1 sec. stimulation at maximum frequency at about 16·9° C. From the average initial heat per g. per sec. for stimuli of 90 per sec. recorded in the last section, the value for maximum frequency can be obtained by extrapolation on the frequency curve; it is $58{\cdot}5 \times 10^{-6}$ cal./g. sec. This number divided by 80 gives $0{\cdot}73 \times 10^{-6}$ cal./g. as the initial heat per isolated impulse at about 16·5° C. For frog's nerve Hill gives 0·26 microcal./g. and 0·067 microcal./g. as the values of initial heat per single isolated impulse at 0° C. and 20° C. respectively. Thus the initial heat due to a single isolated impulse of crab's nerve at about 16·5° C. is about three times as great as that of frog's nerve at 0° C. and at least ten times as great as at 20° C.

We have no data for calculating the impulse heat at lower temperatures with the same accuracy as for 16·5° C. From the size, however, of comparable records of 1 or 2 sec. stimuli at such low frequencies as 6, 10 or 16 per sec., given by nerves at 16° C. and at 10° C., we feel sure that the initial heat per single impulse is not smaller at 10° C. than at 16·5° C., but, if anything, greater.

It may be of interest to note that we have obtained with a single Zc galvanometer, without amplification, records of heat production due to five isolated impulses of crab's nerves, measuring as much as 5 mm. in height. With a good thermostat and amplification it should be possible to register and measure directly the heat production due to a single isolated impulse of crab's nerve.

(4) *The ratio of initial heat to total heat.*

Hill, in his original investigation [1929] of the heat production of crab's nerve, stated that the initial heat is about 2½ p.c. of the total heat. We had in the course of our work three experiments regarding this point (Table III).

In determining the total heat lasting over half an hour or so, the difficulty lies in the creeping of the base line, and one often feels at a loss

TABLE III.

No. of exp.	Temp. °C.	Stimulus: Duration sec.	Stimulus: Frequency shocks per sec.	Total heat 10^{-4} cal./g.	Initial heat 10^{-4} cal./g.	P.c. of total heat
1	19·3	4	92·0	7330	145	2·0
2	20·0	4	92·0	15500	180	1·2
3	18·0	170	6·7	25800	718	2·0

as to how the latter should be drawn. Of the three experiments above, No. 1 was worst and No. 2 best in this respect. In No. 3 the duration of stimulus was 3 min., so there is a good deal of uncertainty in the estimation of initial heat as well. It is probable that Hill's value is somewhat too high, since his "initial" heat, determined by analysis in 5 or 6 sec. units, must have included some recovery heat.

(5) *Short stimuli in nitrogen.*

The distinctness of initial and recovery heats in crab's nerve offers a favourable opportunity for testing the question of their possible separation by means of oxygen want. The difficulty is that crab's nerve is incapable of standing any considerable amount of stimulation in nitrogen. Fig. 10 gives some idea of the rapidity with which it fails. In curve *A*, the four 4 sec. stimuli at 127 per sec. were all the stimulation given after introduction of nitrogen; similarly, in curve *B*, the total amount of stimulation in nitrogen was the five 4 sec. stimuli at 92 per sec. plotted. Yet in half an hour's time the nerve had failed completely. At rest, however, in nitrogen the nerve is able to keep its excitability much longer, and it is generally possible to obtain one or two good records in nitrogen for analysis.

Fig. 11 shows the record of a 4 sec. stimulus at 92 per sec. in nitrogen at 17° C. and its analysis. In this experiment nitrogen was introduced immediately after sucking out the sea water, and the record shown was taken 46 min. afterwards as the first thing in the experiment. It is considerably smaller than records usually are in oxygen, but its analysis is indistinguishable in shape. Not only are there (*a*) the usual large outburst of initial heat during the stimulus, (*b*) the abrupt fall when the stimulus ends, and (*c*) the slow rising of recovery heat to a maximum, but the ratio of the maximum recovery heat rate to the total initial heat, namely 12 p.c., is the same as the average for oxygen experiments at about this temperature. As in frog's nerve, therefore, oxygen want merely alters the size but not the course of heat formation due to stimulation.

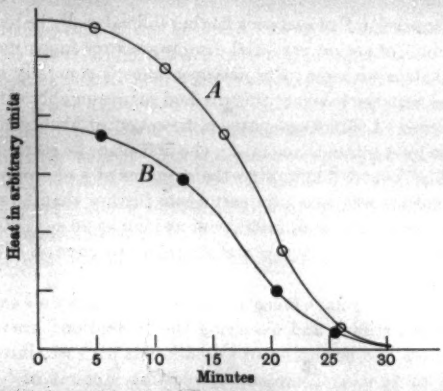

Fig. 10. The rapid failure of crab's nerve when stimulated in nitrogen. The points plotted represent the size of heat response to a 4 sec. stimulus at various times after the admission of nitrogen and include all the stimuli given in this gas. In both *A* and *B* complete failure occurred in about half an hour.

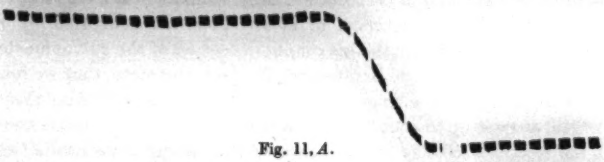

Fig. 11, *A*.

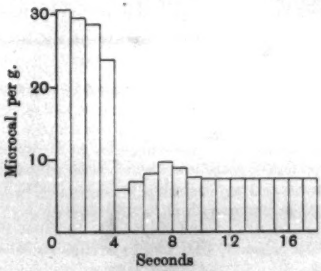

Fig. 11, *B*.

Fig. 11. *A*, record of a 4 sec. stimulus, 92 per sec., 46 min. after introduction of nitrogen, at 17° C., nerve having received no stimulation previously. *B*, analysis of same record. Though somewhat smaller in size it is otherwise indistinguishable from typical analyses in oxygen due to similar stimuli.

In this connection Furusawa's finding will naturally be recalled, that the "retention" of action potential disappears very much more rapidly in oxygen than in nitrogen; this disappearance is generally assumed to be associated with the recovery process, and so presumably with recovery heat production. A direct comparison, however, of his finding with our heat analysis we hesitate to make. In the first place he started observing his "retention" curves 1 min. after the stimulus at ½ or 1 min. intervals, while our analysis was generally carried no further than 20 sec. In the second place his results were for stimuli as long as 20 sec., 50 make and 50 break shocks per sec., while the stimulus we gave in nitrogen was always 4 sec.

Cowan and Feng have recently repeated Furusawa's experiments, using 2 to 4 sec. stimuli and observing the "retention" curves starting 3 or 6 sec. from the beginning of the stimulus at 6 sec. intervals for a period of 8 to 10 min. Their curves plotting amount of "retention" against time from the beginning of stimulus from 1 min. onwards confirm Furusawa in that the oxygen curve always falls much more steeply. During the first half minute or so, however, there is hardly any difference between nitrogen and oxygen curves. Any ordinate of the curve in that time, expressed as a fraction of action potential generated by the stimulus (represented by the maximum deflection of the galvanometer), is the same in oxygen and in nitrogen. The fact, therefore, that we found the heat analysis in nitrogen to be entirely unchanged from that in oxygen, at least up to about 20 sec., is not in conflict with what is known of the electrical change of the nerve in the two gaseous media, even though a strict correlation between the recovery heat production and the restoration of the initial polarized state of the nerve be assumed[1].

[1] A strict relation between the two is not borne out by experimental results. Cowan and Feng observed that about 86 p.c. of the action potential or depolarization caused by a 2 sec. stimulus disappears at the end of 6 sec., in which time the recovery heat production due to the stimulus can hardly have been completed to the extent of more than 1 p.c. Further, the total disappearance of the retention of action potential after a short stimulus of 2 to 4 sec. requires less than 2 min. according to Levin [1927]. This is rather short and possibly complicated by the gradual drift of injury potential. Cowan and Feng, taking precautions to allow for such drift, found the time to be about 10 min. at about 17° C. Recovery heat production, however, after 2 to 4 sec. stimulus, is certainly not completed in less than 20 min. Clearly some mechanism must exist in the nerve which effects an immediate restoration, more or less complete, of its working condition after stimulation. While ultimately the energetic processes testified by the recovery heat production are perhaps responsible for the restoration of the original polarized state of the nerve after a stimulus, between such processes and the actual event of restoration there must be essential links whose nature so far eludes us.

It is possible that if the heat analysis were carried on for several minutes after the stimulus the divergence in nitrogen and oxygen would become apparent, just as in the case of action potential retention reported by Furusawa and confirmed by Cowan and Feng. Because of the technical difficulty, however, of working with nerves in nitrogen over longer periods we have not put this point to experimental test.

There are, however, conditions under which asphyxiation, while leaving the general course of heat production unaffected, does appear to change the quantitative relation between recovery heat rate and the total initial heat that went before. Fig. 12, *A* and *B*, are analyses of two

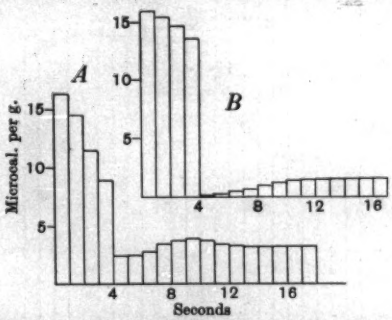

Fig. 12. Analyses of 4 sec. stimuli in nitrogen. *A*, 92 per sec. at 19·3° C., *B*, 44 per sec. at 11·0° C. In both cases the nerves had been partially exhausted previously. Note that the maximum recovery heat relatively to the total initial heat preceding is much lower in these two instances than usual for responses in oxygen.

4 sec. stimuli in nitrogen from two separate experiments at 19·3° C. and 11·0° C. respectively. Both are from partially exhausted nerves. Before *A* three 4 sec. stimuli in oxygen and another three 4 sec. stimuli after the introduction of nitrogen had been taken. *B* was the first stimulus 1 hour after the admission of nitrogen, but before the introduction of nitrogen the nerve had received five 4 sec. stimuli and seventeen 2 sec. stimuli at various frequencies. The maximum recovery heat rate in *A* was 8·5 p.c. of the total initial heat preceding as against 16 p.c., the average value for stimuli in oxygen at a similar temperature. The maximum was also reached somewhat later in time. In *B* the recovery process was so slow that the maximum did not seem to be quite reached even at the thirteenth second after the stimulus. Assuming the highest recovery rate, at the

end of the analysis shown, to be the maximum, it is about 3·1 p.c. of total initial heat, while 5·8 p.c. is the average for oxygen responses at the same temperature. The oxygen reserve of such partially exhausted nerves must be rather low. It is conceivable that the recovery heat rate, while primarily depending upon the amount of initial breakdown, adjusts itself somehow to the existing level of oxygen reserve, and that a depleted oxygen reserve affects the initial and recovery heat production somewhat disproportionately, the latter being relatively more affected.

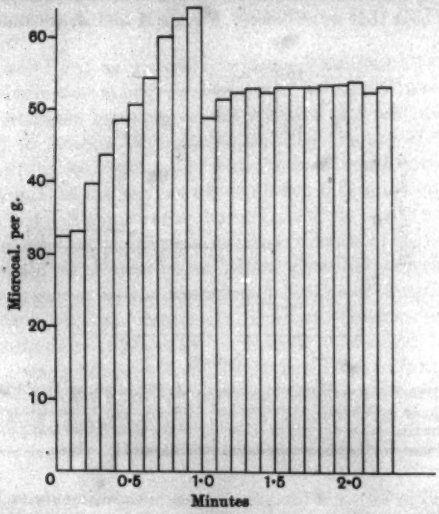

Fig. 13. Analysis of heat from 1 min. stimulus, 6·7 per sec. at 10·5° C., showing that at sufficiently low frequencies crab's nerve may be stimulated as long as 1 min. without failure.

Prolonged stimulation.

Crab's nerve, while very fatigable, is not incapable of prolonged activity, provided it be stimulated at a sufficiently low frequency. In Fig. 13 is the analysis of a 1 min. stimulus at 6·7 per sec. The analysis was done by 6 sec. units with zero remainders. No attempt was made to smooth out the slight oscillations. It is clear that no significant failure occurs during 1 min. stimulation at this frequency. In Fig. 14 is shown (*A*)

a 91 min. stimulus at 4·3 per sec. and (*B*) a 130 min. at 1·2 per sec. In *A*, although a certain amount of failure is obvious in the course of stimulation, it is not much. In *B* no serious failure is discernible after 2 hours 10 min. continuous stimulation, except for the rather uncertain creep of the base line. What failure there was may well have been due to spontaneous deterioration of the nerve rather than to stimulation. A steady state of heat production is thus apparently possible in crab's nerve if

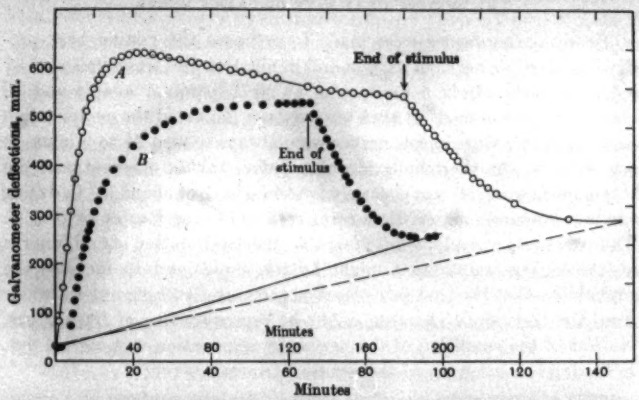

Fig. 14. Galvanometer deflections on scale for *A*, 91 min. continuous stimulation at 4·3 per sec.; *B*, 2 hr. 10 min. continuous stimulation at 1·2 per sec.; both at 19·5° C. Abscissa, inner scale for (*B*), outer for (*A*); ordinate, same for both. It is evident that crab's nerve is capable of keeping up prolonged activity at very low frequencies.

stimulated at the proper frequency, and the natural capacity of crab's muscle for prolonged steady contraction must depend upon the fact that, after a short preliminary period during which a high frequency is necessary, one or two impulses per second coming from its nerve are sufficient to keep it in tetanic contraction.

It is interesting to calculate the ratio of total heat to initial heat in such an extremely prolonged stimulus as shown in Fig. 14, *B*. Since there is no definite sign of failure in the course of stimulation, it may be assumed that each stimulus continues to call forth a full-sized impulse. From a preceding section, the initial heat per single isolated impulse is 0·73 microcal./g. At a frequency of 1·2 per sec. the impulse set up may be considered as practically isolated. Hence it may be calculated that the

total initial heat in the 130 min. stimulation is 6830×10^{-6} cal./g. The total heat can be estimated from the total area under the curve and above the base line, as drawn in Fig. 14, *B*. It is 518,000 microcal./g. So the ratio of the total heat to initial heat is 76. While this number is only an estimate, it is remarkable that it comes to be of the same order as that more directly determined in a short stimulus.

The resting heat rate of crab's nerve.

Several experiments were made to estimate the resting heat production of crab's nerve in oxygen and in nitrogen and when poisoned by iodoacetic acid. Only a thermostat at 20·7° C. was available and all observations were made at that temperature. To avoid the complication of a vapour pressure effect, nerves were always soaked 2½ to 3 hours in sea water in the thermopile chamber before taking observations; the thermopile, however, was generally kept in a bath of about 14° C. during the first 2 hours' soaking, then tranferred to the thermostat at 20·7° C. This procedure was adopted for fear that the deterioration of crab's nerve at the higher temperature might be too rapid, and to increase the probability that the heat rate observed soon after sucking out sea water from the thermopile chamber might be representative of fresh nerve. No test of the condition of the nerves by stimulation was carried out. Following is a summary of the results on normal nerve:

Table IV. Resting heat rate in cal./g. hour.

No. of exp.	In oxygen	After admission of nitrogen (hours) 1	2	3	4	5	6
1	0·72	0·23	0·15	—	—	—	—
2	0·83	0·30	0·22	0·17	0·13	0·11	0·10
3	0·65	0·27	0·19	0·14	0·12	—	—
4	0·53	—	0·13	0·17	—	—	—
5	0·74						
Average	0·69						

According to Meyerhof and Schulz, the oxygen consumption of crab's nerve at rest at 16° C. is 0·8 c.c. per g. of dry weight per hour, or about 96 c.mm. per g. of moist tissue per hour, assuming a water content of 88 p.c. for these nerves. This may be considered to be equal to a heat liberation at the rate of 0·48 cal./g. hour. Assuming a $Q_{10} = 2{\cdot}5$, the corresponding value at 20·7° C. is 0·74 cal./g. hour, very near to our average value of 0·69 cal./g. hour found by direct measurement.

On the basis of the recent measurement by Beresina [1932] the

resting heat rate of crab's nerve in oxygen is about three times as great as that of frog's nerve under similar conditions.

The resting heat production of crab's nerve drops relatively very sharply on replacing oxygen with nitrogen. After 1 hour it is reduced to about 25 p.c. of the oxygen value; after 2 hours to under 20 p.c.; then occasionally remaining approximately constant or more often increasing slowly. In the case of frog's nerve it takes 2½ hours to reduce the resting heat rate in nitrogen to 20 to 25 p.c. of that in oxygen; once reduced to this lower level it apparently remains constant for hours [Beresina, 1932]. Crab's nerves contrast with frog's nerves in respect to both these features. Perhaps the more rapid fall of the resting heat rate of crab's nerve in nitrogen may be related to its earlier onset of inexcitability when subjected to oxygen want, particularly when it receives at the same time any amount of stimulation. It seems that the oxygen reserve in crab's nerve is smaller: perhaps not per g. of nerve but relatively to the fact that the resting heat rate of crab's nerve is about three times as large as that of frog's and that the total heat liberation (initial plus recovery) due to a short stimulus more than thirty times as great. The fact that a constant level of resting heat production of crab's nerve in nitrogen is seldom maintained probably means that the rate of glycolysis which is presumably largely responsible for the anaerobic resting heat begins to fall earlier and more rapidly in this nerve than in frog's.

Holmes [1929] has investigated the change of glycogen content and lactic acid production of crab's nerve resting in oxygen and in nitrogen. As emphasized, however, by the author himself, his results show extreme variations, and no significant average could be taken. Correlation of his results and ours, therefore, is hardly possible.

The effect of iodoacetic acid on crab's nerve is far more pronounced than on frog's. The latter requires 2 hours or longer soaking in 0·4 p.c. iodoacetate to produce conspicuous poisoning [Feng, 1932], while 40 min. soaking in 0·05 p.c. iodoacetate for the former already considerably diminishes its response to a short stimulus, and very greatly decreases its ability to stand further stimulation. For resting heat study, where soaking cannot safely be less than 2 hours because of the vapour pressure complication, two concentrations of iodoacetate were tried, 1/4000 and 1/8000 in sea water. The stronger concentration in all the four experiments made with it caused the initial level of resting heat rate in oxygen to be somewhat lower than in normal nerves; the more striking effect, however, was that even this lower level was not maintained but progressively diminished to practically nothing or became negative in 3 to 5

hours. With the weaker concentration also one generally found a slightly lower initial rate of heat production which, however, remained approximately steady at least for 1 hour or so. The average of four experiments gave 0·56 cal./g. hour as the resting heat rate in oxygen of crab's nerves poisoned by 1/8000 iodoacetate. On introducing nitrogen the fall of heat rate was much quicker than in normal nerves. In about 2 hours' time it was practically nothing, and became negative when observation was further continued. Here, again, we have negative heat production. While it is not difficult to conceive that, in a strongly poisoned nerve, where both respiration and glycolysis are completely or nearly inhibited, the resting heat production as observed may be nothing, one cannot easily imagine how negative heat production could occur, apart from technical complications, *e.g.* of a galvanic effect due to imperfect insulation. The point is not yet clear.

Discussion.

Hill showed that the course of the recovery heat production in frog's nerve can be represented by a double exponential of the form

$$Y = Ae^{-at} + Be^{-bt}.$$

The application of such an equation to the recovery heat production of crab's nerve is out of the question, for here the recovery heat rate, instead of starting out from its highest value immediately after the passing of an impulse, as required by the equation, begins low and gradually rises to a maximum. Such a time course would require for its representation an equation of the type

$$Y = Ae^{-at} - Be^{-bt}.$$

Like many natural processes, indeed like the recovery process in muscle [Hartree, 1932], the recovery process in nerve gradually works up to its maximum and then declines.

One of the more striking characteristics of crab's nerve is its great susceptibility to fatigue when stimulated even at very moderate frequencies. While no answer is possible to the question why frog's nerve is so resistant to fatigue while crab's nerve is so susceptible, it may be relevant to note that the recovery heat production after a stimulus starts out at its maximum rate in frog's nerve, and takes a much quicker course than in crab's nerve, in which the maximum recovery rate is not reached until some seconds after the stimulus is over. It is possible that fatigability is due to slow recovery, testified by a slower recovery heat production. At any rate at lower temperatures where recovery is further

slowed down, the fatigability of crab's nerve becomes more pronounced. At 0° C. even frog's nerve shows evident signs of fatigability in a stimulus as short as 16 sec. at 30 to 40 per sec. [Hill, 1932].

Now fatigability is also a characteristic of the central nervous system. May we not suspect conversely that, if we could get a workable preparation of grey matter for neurothermic study, we could find that here too the course of the recovery heat production after a stimulus was slow, very much more similar to that in crab's than in frog's nerve?

Recently there has appeared an investigation by Hoffmann, Holzlöhner and Leegaad [1932] on the heat production of the surviving spinal cord of the frog. They report that for durations of stimulus from 1·3 to 19·5 sec. at 21 to 22° C. the total heat production per sec. of stimulation per g. wet weight of the spinal cord is 580 to 2324 microcal.; values comparable to those given by crab's nerves. According to them, however, all this heat was completed within 2 to 5·4 min. Further they present analyses showing that the heat rate after the stimulus may be even somewhat greater than during the stimulus; that in a stimulus of about 5 sec. the average heat rate in the 15 sec. or so following the stimulus is nearly as high as that during the stimulus. Unfortunately they do not discuss either of these unusual features or the possibility of after discharge following the stimulus. Their work, as it stands, does not support any correlation between fatigability and low rate of recovery heat production in the case of the central nervous system such as seems to exist in crab's nerve.

Summary.

1. The heat production of crab's limb nerve has been more fully investigated than in Hill's original work on the subject.

2. The optimal stimulus for this nerve at about 17° C. has an *RF* (resistance in ohms × capacity in μF, which is proportional to time of discharge) of about 1200, which is six times as great as for frog's nerve.

3. The effect of frequency of stimulation has been determined, the maximum response being obtained at a frequency (at about 16·9° C.) in the neighbourhood of 200 per sec.

4. Analysis of photographic records of heat production due to short stimuli in oxygen and nitrogen was carried out in 1 sec. units. The separation of initial and recovery phases is very distinct; the maximum of recovery heat rate is not reached until some seconds after the stimulus is over, so that there is always a very abrupt fall of heat rate at the end of a stimulus and always a "hump" in the recovery part of the analysis.

5. The course of the heat production of fresh crustacean nerve in nitrogen is exactly the same as in oxygen. Nerves, however, which have previously been partially exhausted have a lower recovery heat rate, relatively to the total initial heat going before.

6. The initial heat due to nearly maximal stimulation both with respect to strength and frequency is found to be 58×10^{-6} cal./g. sec.: this is $1\frac{1}{2}$ to $2\frac{1}{2}$ p.c. of the total heat (initial plus recovery).

7. The initial heat per single isolated impulse is about 0·73 micro-cal./g. at 16·5° C.

8. While stimulated even at frequencies only about a quarter or so of the maximum to which it will respond, crab's nerve is shown to be very fatigable; it is quite capable, however, of maintaining prolonged activity if the frequency of stimulation is very low. It shows no distinct failure when stimulated at 1·2 per sec. for as long as 2 hours.

9. The resting heat production of crab's nerve in oxygen at 20·7° C. is about 0·69 cal./g. hour, agreeing satisfactorily with the oxygen consumption measurement of Meyerhof and Schulz. Some observations of resting heat production of nerves in nitrogen and when poisoned by iodoacetic acid are recorded.

The authors owe deep gratitude to Prof. A. V. Hill who suggested this work and whose aid and encouragement kept it going. To Dr E. J. Allen and the staff of the Plymouth Laboratory they wish to express their sincere thanks for kind hospitality which made their stay so enjoyable; to Mr S. L. Cowan they are indebted for much willing assistance as well as pleasant discussion in the course of their work.

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THE LOCALIZATION OF THE ACTION OF DRUGS ON THE PULMONARY VESSELS OF DOGS AND CATS.

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Since previous studies of the effect of drugs on the pulmonary vessels have been reviewed by Wiggers [1921], Tigerstedt [1923] and Daly [1932], it is not necessary for us to present a connected historical survey. We will discuss earlier work only in so far as it bears directly on our own experiments, which were devised with the chief object of obtaining information regarding the site of action on the pulmonary circulation of various pharmacologically active substances which are found in tissue extracts.

In early work on this subject the assumption was usually made that any observed effects of drugs on the resistance of the pulmonary vessels to the flow of blood were due to an action on the pulmonary arterioles. The suggestion that changes in the tone of the pulmonary veins might appreciably affect the resistance originated partly from the study of the action of drugs on isolated veins [Inchley, 1923, 1926; Franklin, 1932, who gives other references], and partly from observations of the lung volume when the circulation was intact [Luisada, 1928; Mautner and Pick, 1929]. Mautner and Pick have shown that the blood flow through the portal system of the dog is much influenced by the resistance offered by the hepatic veins under the action of histamine, and they give evidence suggesting that histamine also causes the pulmonary veins to constrict.

The conclusions of Mautner and Pick regarding the portal circulation have been confirmed and extended in an investigation carried out in this laboratory [Bauer, Dale, Poulsson and Richards, 1932]. The present work was undertaken with the object of applying similar methods to the study of the behaviour of the pulmonary vessels. It was hoped that fresh light might be thrown on the importance of the pulmonary veins by

recording the changes in the lung volume as well as other effects during perfusion.

The most widely used method of studying the effect of drugs on the pulmonary vessels is that introduced by Brodie and Dixon [1904], in which the lungs are perfused with blood under constant pressure and the effect on the outflow is recorded. Under these conditions the effect of a vaso-constrictor drug is seen in a diminution of outflow, and some information regarding the main site of the effect may be obtained by recording the volume of the lung. If the effect is predominantly on the inflow to the lung the volume of the lung will be diminished; if it is predominantly on the outflow the volume will be increased. Analogous conclusions may also be drawn regarding the action of vaso-dilator drugs.

In another less commonly used method of perfusing the lungs, the pulmonary artery is connected directly to the perfusion pump, so that the rate of flow is approximately constant and the effects of the drugs are seen in changes in the pressure in the pulmonary artery. If the pump were completely rigid these changes of pressure would not affect its output. Under these conditions, a vaso-motor drug would have practically no effect on the volume of the lung, except in so far as it acted on the veins.

The apparatus which we have used was so devised that the perfusion could be carried out either under conditions of approximately constant pressure, or under conditions of approximately constant flow. In the former case, changes in the volume of the lung were partly due to changes in the resistance to inflow and partly due to changes in the resistance to outflow. In the latter, the effect on the volume of changes in the resistance to inflow was largely eliminated, and effects due to changes in the resistance to outflow were better shown. By changing repeatedly from one method of perfusion to the other, it has been possible further to test the conclusions suggested by the observed effects of drugs. The two methods of perfusion are easily distinguished in the figures, since when the pump is connected directly to the artery the record of the inflow pressure shows large oscillations with the rhythm of the pump, which are absent when the other method of perfusion is used.

The effects shown in our records of lung volume are presumably due to changes in the volume of blood in the capillaries, and it is possible that they are caused in some cases by changes in the tone of these vessels, though we know of no evidence that such changes can occur in the lungs. Constriction of the capillaries would produce an increase in resistance and a decrease in the lung volume, and would thus be indistinguishable in our experiments from constriction of the arterioles, except in so far as the

constriction of capillaries might cause a transient increase in outflow owing to their large blood capacity. When such a combination of effects has occurred, we have been unable to decide whether the effect should be attributed to an action on the arterioles or on the capillaries, and we have been content to consider the two kinds of vessel together and to attribute such effects to an action on the "inflow."

Methods.

The animals (dogs and cats) were anæsthetized with ether and bled through the carotid artery. The blood was either defibrinated or collected in a bowl containing heparine. In experiments with cats, about 50 c.c. of Ringer's solution were usually injected into the jugular vein, so that sufficient blood to fill the apparatus (at least 120 c.c.) was obtained from a single animal. The lungs were then removed from the animal, the arterial cannula was inserted in the pulmonary artery through the wall of the right ventricle, and the venous cannula was inserted through the left auricular appendix as deeply as possible without obstructing the flow, so as to reduce to a minimum possible passive changes in the volume of the auricle. A thread was tied round the heart at the base of the ventricles, in order to prevent blood from escaping from the left auricle through the mitral valve. The bronchial vessels were not perfused.

The lungs were enclosed in the plethysmograph described by Bauer, Dale, Poulsson and Richards [1932], and their volume was recorded by means of a Brodie bellows [1902] attached to this plethysmograph. A tube, which was tied into the trachea, passed to the outside of the plethysmograph and was closed with a clip. It is known that the distension of the lungs by negative pressure applied to the pleural surface decreases the resistance of the pulmonary vascular bed [Daly, 1930], but if the distension is produced by a positive pressure in the trachea the resistance may be increased. As it was desirable to reduce the proportion of the resistance due to simple mechanical factors, the lungs were perfused in an almost completely deflated condition.

Conditions resembling more closely those occurring in the intact animal would have been obtained if the lungs had been rhythmically inflated during the experiment. Under these conditions, however, it would have been difficult to distinguish effects on the average volume due to constriction of the bronchi from those due to changes in the blood content of the lungs. We believe that, under the conditions we have chosen, it is unlikely that changes in the tone of the bronchi will affect the record.

In order to convince ourselves that the changes which we were recording did really represent changes in the blood content of the lung, we have performed a control experiment in which we used an elegant method introduced by Daly [1930]. Daly recorded changes in the volume of blood in perfused lungs by recording the corresponding changes in the volume of blood outside the lungs in the apparatus. In most of our experiments we did not use this method because of the difficulty of combining it with a record of the outflow. In our control experiment, the pulmonary artery of a dog was connected directly to the pump and the outflow was not recorded. The plethysmographic record was compared with the record of the volume of blood in the venous reservoir. It was found that occlusion of the inflow or outflow, or the injection of adrenaline histamine or acetylcholine, produced similar but opposite, immediate effects on the two records. The plethysmographic record was slightly less sensitive than the other record and was more liable to show changes unconnected with the injection of drugs, which were probably due to small changes in the temperature of the plethysmograph. We concluded that records obtained in this way would give us a reliable indication of the main site of action of drugs, and enable us to record the rate of outflow from the veins as well as the volume of blood in the lung.

A bath containing water thermostatically kept at 40° was placed about 15–25 cm. above the level of the lungs. This bath contained a spiral glass coil, a pump [Dale and Schuster, 1928] and an arterial reservoir for blood (Fig. 1). Blood from the venous reservoir first passed through the coil and then the pump. After leaving the pump it could be shunted by means of artery forceps applied to rubber connections, so that it either passed directly into the pulmonary artery or else into the arterial reservoir and thence to the pulmonary artery. The level of the blood in the arterial reservoir was kept constant by means of an overflow tube to the venous reservoir. A vertical glass tube was connected to the arterial supply by means of a T-piece. The height to which the blood rose in this tube gave a measure of the arterial pressure, and this was recorded by means of a small Brodie bellows connected to the upper end of the vertical tube. The venous outflow was recorded by the method described by Gaddum [1929], and the blood was collected in a venous reservoir which was open to the air. The outflow recorder was at about the same level as the auricle.

The apparatus was originally designed for use with cats, and our records show that when cats' lungs were perfused the apparatus was effective in maintaining either the perfusion pressure, or the rate of flow,

constant. When the same apparatus was used to perfuse dogs' lungs, it was found to be too small. On the one hand, the pump was not large enough to maintain so great a flow constant against varying pressures, and on the other, the tubing between the arterial reservoir and the cannula was not wide enough to ensure that the pressure in the cannula was quite independent of the rate of flow. Thus, in Fig. 5 it will be seen that histamine produced a small rise of perfusion pressure and a small fall of outflow even when the blood was so shunted as to maintain these quantities as nearly as possible constant. If a larger apparatus had been used, it would probably have been possible to demonstrate a more striking contrast between the two methods of perfusion in experiments on the dogs' lungs, but since the contrast was already sufficiently definite for our purpose we did not construct another apparatus. In some cases, when the dog was a large one, we have tied off one lung at its hilum and perfused the other.

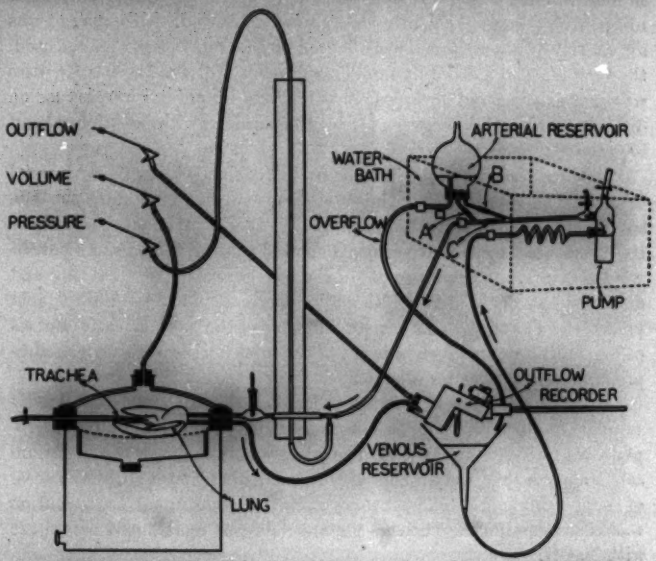

Fig. 1. When *A* and *B* are clamped with artery forceps, and *C* is open, the artery is connected directly to the pump. When *C* is clamped, and *A* and *B* are open, the perfusion pressure is approximately constant.

Two types of difficulty were encountered in experiments with cats. In the first place, it sometimes happened that the vessels rapidly acquired such a marked tone that the blood almost ceased to flow. In such cases, the addition of adrenaline to the blood was found to relax the tone and to allow the experiment to be completed [cf. Löhr, 1923]. In the second place, the plethysmographic record frequently showed a steady increase in lung volume, which was so marked as to prevent records being taken until more than an hour after the perfusion started. This change was apparently due to the use of defibrinated blood. When heparine was used, the lung reached a steady volume much more rapidly. These difficulties were not encountered in experiments on dogs' lungs, and in this case no difference was noticed between the results obtained with defibrinated and heparinized blood.

The drugs were injected into the rubber tubing leading to the arterial cannula. The volume of fluid injected was between 0·05 and 0·2 c.c. The solution of adrenaline used in most of the experiments contained chloretone, but a few control experiments showed that all the effects recorded were also produced by a sample of the base prepared synthetically and dissolved by the aid of HCl. Histamine was used in the form of the acid phosphate, but the doses are given in terms of the base. The doses of all other substances are given in terms of the total weight of the salt used to prepare the solutions for injection.

We wish to express our thanks to Dr K. Lohmann who very kindly supplied us with the specimens of muscle adenylic acid and adenylpyrophosphate which we have used. The latter was in the form of the barium salt. This was dissolved with the aid of a small quantity of HCl. An excess of sodium sulphate was added to precipitate the barium and the solution was then centrifuged. The supernatant fluid was neutralized before use with NaOH.

Results.

Adrenaline.

Dogs. In confirmation of much previous work, we find that adrenaline normally increases the resistance of the dog's pulmonary vessels to the flow of the blood. This effect is accompanied, as shown in Fig. 2 *a*, by a fall in the lung volume, and is thus mainly due to the constriction of the inflow. This conclusion is confirmed by the fact, shown in Fig. 2 *b*, that when the rate of inflow is made more nearly constant by connecting the pulmonary artery directly to the pump the effect on the volume is diminished. In two experiments out of ten, the substitution of a direct

perfusion by the pump for the perfusion under constant pressure actually caused a reversal of the effect on the volume, which was now increased under the action of adrenaline (cf. Fig. 5). Though the main action of adrenaline in this experiment was on the inflow, the drug appeared therefore to have also a constrictor action on the outflow.

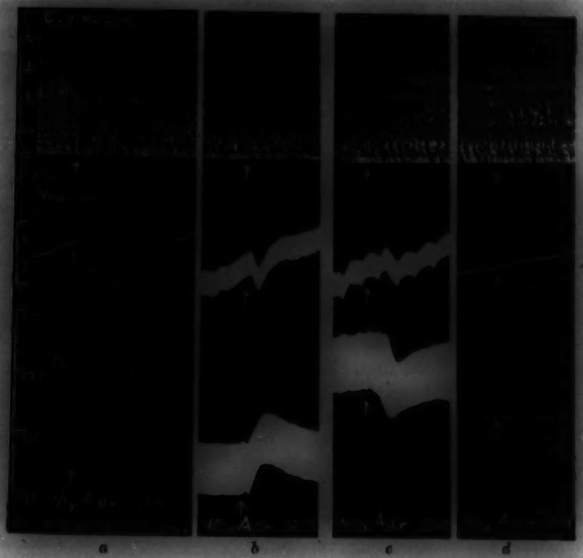

Fig. 2. Dog. Adrenaline (+ chloretone). Outflow. Lung volume. Perfusion pressure (mm. of blood). At 12.16, 1 mg. of ergotoxine ethane sulphonate was added to the blood in the venous reservoir.

Fig. 3 shows another effect which adrenaline sometimes produced. In this record, the second injection of 25γ of adrenaline is seen to cause a slight rise in the perfusion pressure. This would not be sufficient appreciably to affect the output of the pump, but the effect, if any, would be to cause a diminution of the rate of flow of blood into the lungs. Nevertheless, the rate of outflow was definitely increased. Under these circumstances the effect on the volume of the lung was inevitably a fall. This increase of outflow was clearly shown when the pump was connected

directly to the pulmonary artery (Fig. 3 *b*), but not when the lungs were perfused at constant pressure (Fig. 3 *a*). This was presumably because the rate of inflow fell in the latter case, owing to increase of the inflow resistance, while it was maintained practically constant in the former case. Adrenaline certainly produced a restriction of the inflow in this experiment, but the record of the rate of outflow shows that it had other effects as well. We do not know whether this increase of outflow was due to dilatation of the pulmonary veins letting the blood flow out, or to constriction of the capillaries forcing it out.

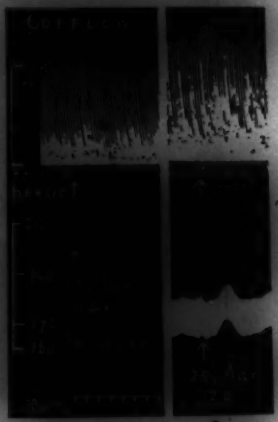

Fig. 3. Dog. Adrenaline (chloretone-free). Outflow. Perfusion pressure (mm. of blood).

The effect which has just been described is the only evidence we have obtained which might be interpreted as indicating that a drug was having an action on the outflow contrary to its action on the inflow. When this phenomenon was first observed, it seemed possible that adrenaline had a specific acion in releasing blood from the pulmonary circulation, just as it has been found to release blood from the portal circulation. Fig. 3 shows the most striking example of this phenomenon which we have seen. This effect was only seen in five out of ten experiments, and, as has already been mentioned, two experiments provided definite evidence that adrenaline was constricting the pulmonary veins. We have not been able to determine what factors govern the direction of the action of adrenaline on the pulmonary veins.

The constrictor effect of adrenaline is reversed by ergotoxine. In the experiment shown in Fig. 2, 1 mg. of ergotoxine ethane sulphonate was added at 12.16 to the blood in the venous reservoir. This produced a reversal of the effect of adrenaline on the perfusion pressure, while the direction of the effect on the volume was unaltered. The main effect of this dose of adrenaline was now dilator, and, since it still produced a fall in the lung volume, the effect was mainly on the outflow. This conclusion is supported by the fact that, after ergotoxine, the effect on the volume was best shown when the lungs were connected directly to the pump, so that the rate of flow of blood into the lungs was kept nearly constant.

When the lung was perfused at constant pressure, the increase in the rate of outflow, which was slight but just visible on the outflow record, was compensated by an increased rate of inflow, so that no effect on the volume was produced. When the lungs were connected directly to the pump, this increase in the rate of inflow could not occur.

In another experiment the vaso-dilator action of adrenaline after ergotoxine was accompanied by an increase in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure. In this experiment the dilatation must have been mostly due to an effect on the inflow.

Cats. It is well known that adrenaline may, under different conditions, produce either constriction or dilatation of the pulmonary vessels of the cat. The factors controlling the direction of the effect have been studied by Tribe [1914], and we are able, in general, to confirm her conclusions. In the early part of the experiments, the injection of small doses of adrenaline has invariably caused a large and prolonged vaso-dilatation. This effect is shown in Fig. 4. When the lungs were perfused in this experiment at an approximately constant pressure (Fig. 4 *a*), the injection of 2γ of chloretone-free adrenaline caused an increase in the rate of outflow and no effect on the volume of the lung. When the artery was connected directly to the pump (Fig. 4 *b*), the same injection caused a fall in the perfusion pressure and a small fall in lung volume. The record of outflow shows that the change in the perfusion pressure was not sufficient appreciably to affect the output of the pump. It is clear from the effect of this second injection that adrenaline may produce dilatation of the pulmonary venules in the cat. The fact that adrenaline had no effect on the volume when the perfusion was carried out at constant pressure shows that the pulmonary arterioles were also dilated, and that the increase in the rate of outflow from the lungs was in that case exactly compensated by an increase in the rate of inflow. When the artery was connected directly to the pump, no such increase in the rate of inflow could occur, and the volume of the lungs accordingly fell. Results similar to those shown in Fig. 4 have usually been obtained, but, in some experiments in which adrenaline produced similar evidence of vaso-dilatation by both methods of perfusion, it had no definite effect on the volume of the lung, even when the artery was connected directly to the pump.

In our first experiments, carried out in October, the vaso-dilator action of adrenaline was only seen in the early part of the experiment. The injection of a large dose of adrenaline at this stage caused a very large dilatation. In one experiment, for example, the injection of 0·1 mg. of

adrenaline increased the flow to four or five times its original value, and the vessels never recovered their original tone. In these first experiments, about an hour after the perfusion had started, the injection of small doses of adrenaline ceased to have any effect, and the injection of larger doses (20γ–200γ) caused vaso-constriction. In later experiments, carried out in March, the vaso-dilator action was much more persistent, and, in order to obtain vaso-constriction, it was necessary to use chloretone-free adrenaline,

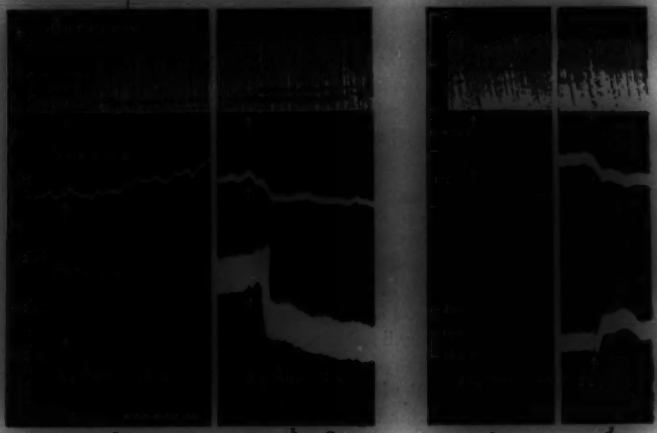

Fig. 4. Cat. Adrenaline (chloretone-free) in two different experiments. Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

so that the constrictor effect of the adrenaline should not be masked by the feeble dilator effect of the chloretone. Fig. 4 *c*, *d* show that this action of adrenaline was mostly due to constriction of the inflow. It was accompanied by a fall in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure (Fig. 4 *c*). When the artery was connected directly to the pump the rate of inflow was artificially maintained almost constant and comparatively little change in lung volume occurred (Fig. 4 *d*). It is possible that the small decrease in volume which did occur under these conditions was partly due to dilatation of the venules.

Histamine.

In their original description of the effect of histamine in increasing the resistance of the pulmonary vessels, Dale and Laidlaw [1910] assumed that the effect was due to an action of the drug on the pulmonary arterioles. Inchley [1923], however, devised a method of perfusing arteries and veins separately with Ringer's solution, and showed that they were both constricted by histamine. Later [Inchley, 1926] he found that isolated

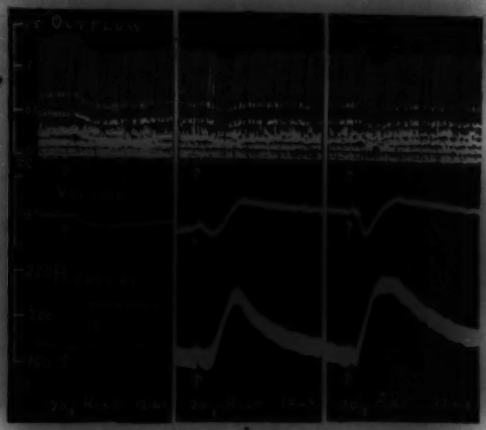

Fig. 5. Dog. Histamine and adrenaline (chloretone-free). Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

veins in general, and the pulmonary veins in particular, were more sensitive to histamine than isolated arteries, and concluded that the effects of histamine were mainly due to an action on the veins. This view was also held by Mautner [1923] and was supported by experiments in which the lung volume of dogs with intact circulation was recorded, together with the pressures in the pulmonary artery and in the left auricle [Luisada, 1928; Mautner and Pick, 1929].

In our experiments, histamine has invariably produced vaso-constriction, both in cats and dogs. When the perfusion was carried out at constant pressure, the fall in blood flow was usually accompanied by a fall in lung volume, and was thus mainly due to constriction of the inflow.

When the artery was connected directly to the pump, this fall in lung volume was followed by a rise which was probably due to the constriction of the outflow. These effects are shown in Fig. 5, taken from an experiment on a dog, and in Fig. 6, taken from an experiment on a cat. In some experiments, larger doses of histamine produced a very large and prolonged rise in lung volume. This effect was not reversible and was probably due to œdema. In the experiments from which tracings are given, the volume

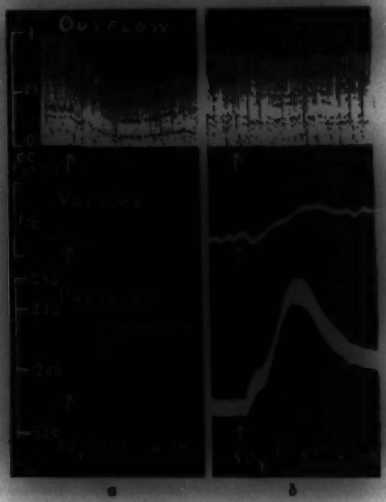

Fig. 6. Cat. Histamine. Outflow. Lung volume. Perfusion pressure (mm. of blood).

showed a tendency to return to its original level, and, though it is possible that histamine caused some œdema in these experiments, the rise in volume cannot have been entirely due to this, and must have been caused, at least in part, by some more easily reversible change.

In Fig. 6 *b* the conclusion that histamine produced constriction of two separate structures is confirmed by the double nature of the rise in perfusion pressure. The first effect of the drug was constriction of the inflow, which caused a rise of perfusion pressure and a small fall of volume. This was rapidly followed by constriction of the outflow, which caused a further rise of perfusion pressure and a simultaneous rise in lung volume. In the

same way the record in Fig. 6 *a* which shows the rate of outflow when the lung was perfused at constant pressure also gives an indication that the drug was acting on two separate structures.

Our results, which were based on experimental procedures which were, in some respects, less drastic than Inchley's, confirm Inchley's main conclusion that histamine constricts both inflow and outflow. These two actions tend to produce contrary effects on the volume and the observed changes in volume are, therefore, small. Under the conditions of our experiments there was no marked and constant difference between the sensitivity of the two kinds of vessels, though in some experiments the arteries appeared to be rather more sensitive than the veins. In this respect our findings differ from those of Inchley.

Acetylcholine.

Acetylcholine has been found to produce vaso-constriction in the pulmonary vessels of rabbits [Antoniazzi, 1931; Euler, 1932] and cats [Hirose, 1932]. In their experiments on dogs Daly and Euler [1932] found that acetylcholine sometimes produced vaso-constriction and sometimes vaso-dilatation. Franklin [1932] found that acetylcholine dilated the dog's pulmonary arteries and constricted the veins. We have found that cats are more sensitive to acetylcholine than dogs.

Cats. The result of a typical experiment is shown in Fig. 7. The outflow records are not included in this figure or in Fig. 8 because they showed no changes. It will be seen that small doses (1–3γ) produced vaso-dilatation, and had no definite effect on the lung volume. After a larger dose (20γ) the initial vaso-dilatation was followed by vaso-constriction and a rise in lung volume. This shows that the pulmonary veins were constricted. In another experiment, the result of which has not been reproduced and in which, when the pump was connected directly to the arterial cannula, acetylcholine (10γ) caused a rise of perfusion pressure and lung volume rather larger than that shown in Fig. 6, it was found that when the perfusion was carried out at constant pressure the same dose caused a diminution of flow and a small and indefinite fall in the volume of the lung. From these results we concluded that these doses of acetylcholine produced constriction of both inflow and outflow in the cat. When the inflow was kept practically constant by the pump, constriction of the outflow caused the rise in lung volume shown in Fig. 7, but, when the perfusion was carried out at constant pressure, the rates of inflow and outflow were both diminished and there was practically no effect on the volume. These effects were all abolished by atropine.

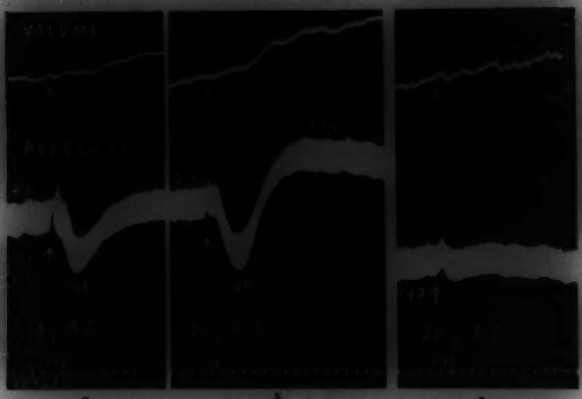

Fig. 7. Cat. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood). At 1.22 1 mg. of atropine sulphate was added to the blood in the venous reservoir.

Fig. 8. Dog. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood).

Dogs. In the experiment shown in Fig. 8 the effects of acetylcholine on the perfusion pressure in a dog's pulmonary artery were similar to the effects seen in experiments with cats, though the doses used were larger. A small dose (10γ, Fig. 8 *a*) caused vaso-dilatation. After larger doses the initial dilatation was followed by vaso-constriction. The dilator action of small doses was not seen in some experiments, but the constrictor action of large doses was always present. The vaso-dilatation was accompanied by a very small increase in lung volume, but, in contrast to the effects seen in cats, the vaso-constriction was always accompanied by a fall in lung volume, even when the pump was connected directly to the pulmonary artery as in the experiment shown in Fig. 8. These effects were increased by eserine and abolished by atropine. In one experiment, eserine converted a vaso-dilator effect into a vaso-constrictor one. This action was presumably due to an increase of the effective concentration of acetylcholine.

It is doubtful whether significance should be attached to the very small rise in lung volume shown in Fig. 8 *a*, *d* after the injection of 10γ of acetylcholine. The direction of this change would indicate that the dilator action of the drug was on the inflow. We can, however, be more confident that large doses of acetylcholine constrict the inflow and have no significant action on the outflow.

Franklin [1932] has recently published an account of the action of acetylcholine on the dog's pulmonary vessels. He found that this drug invariably relaxed isolated rings of pulmonary artery and constricted isolated rings of pulmonary vein, and deduced that it would produce a rise of lung volume. The small dilatation of the lung which followed the injection of 10γ of acetylcholine in the experiment shown in Fig. 8 *a*, *d* is probably the effect which Franklin predicted. It is not difficult to suggest possible explanations of the fact that he did not observe the other more marked effect of larger doses. Franklin's experiments were confined to the trunks of the vessels outside the lungs, and it is improbable that changes in the tone of these large vessels can have much influence on the blood flow. It is possible that acetylcholine has an effect on the small vessels which does not occur in the large ones. Our own failure to obtain evidence of a constrictor action of acetylcholine on the veins was probably due partly to the fact that the effects of such an action would be masked in our experiments by the increase of the inflow resistance, and partly to the fact that the acetylcholine would be mostly destroyed before it reached the veins. In any case acetylcholine was the only drug which never produced any considerable rise in the dog's lung volume in our

experiments, and we consider that it is improbable that the action of acetylcholine on the pulmonary vessels can cause the lungs to dilate appreciably in the whole dog.

Adenosine and its compounds.

Adenosine and its compounds have been shown to be present in a large number of tissues. Some of their pharmacological effects have been studied by Drury and Szent-Györgyi [1929], Bennet and Drury [1931], Zipf [1931], Ostern and Parnas [1932], Deuticke [1932] and Drury [1932]. Zipf has drawn attention to the fact that they are probably responsible for those effects of defibrinated blood which Freund [1920] attributed to the "Frühgift."

We have studied the effects on the pulmonary vessels of adenosine, muscle adenylic acid and adenylpyrophosphate. The latter substance was first isolated from voluntary muscle [Lohmann, 1931]. The effects of all these three substances in our experiments were qualitatively the same, and were in many ways similar to those of acetylcholine. The cat's vessels were much more sensitive than the dog's vessels.

In experiments with cats small doses produced vaso-dilatation, and when the dose was increased the initial dilatation was followed by vaso-constriction. The dilatation was not accompanied by any change in the volume of the lung or outflow and its site could, therefore, not be identified (Fig. 9 *a*, *b*). Fig. 9 *c* shows that, when the lung was perfused at approximately constant pressure, the injection of 0·25 mg. of adenylpyrophosphate caused vaso-constriction, accompanied by a fall in lung volume. This effect must have been due to an action mainly on the inflow. On the other hand, when the pump was connected directly to the artery the initial fall in lung volume was followed by a large and irreversible increase. This second effect may have been due either to prolonged constriction of the outflow or to œdema.

The effects of muscle adenylic acid and of adenosine were indistinguishable from one another when the substances were given in equimolecular doses. Both these substances were, however, much weaker in their action than adenylpyrophosphate. In Fig. 9 *a*, *b* it will be seen that 0·005 mg. of adenosine produced less dilator effect than a dose of adenylpyrophosphate corresponding to 0·005 mg. of its barium salt. Since the ratio of the molecular weights of these substances is 3·3, this result indicates that the addition of the extra phosphate groups to the molecule of adenosine increased its activity more than 3·3 times. In another experiment the constrictor effects of larger doses were compared,

and it was found that each molecule of adenylpyrophosphate was about equivalent in this action to fifteen molecules of adenosine. Since adenylic acid is not more active than adenosine, it is the addition of the pyrophosphate group which leads to the increase of activity. It was, however, found in one experiment that sodium pyrophosphate itself had no action

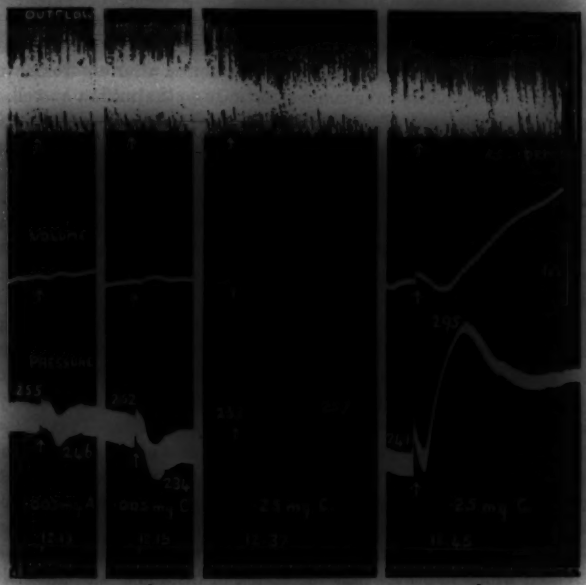

Fig. 9. Cat. *A.* Adenosine. *C.* Adenylpyrophosphate (doses in terms of weight of the barium salt). Outflow. Lung volume. Perfusion pressure (mm. of blood).

when given in comparable doses. In preparing the barium adenylpyrophosphate for injection, an excess of sodium sulphate had been added to ensure complete precipitation of the barium. A control experiment showed that quantities of sodium sulphate, considerably larger than those present in the doses used, had no action in these experiments.

The pulmonary vessels of dogs were much less sensitive, and it was not found possible to inject sufficient of these substances to compare their actions satisfactorily. In one experiment, however, it was found that a

dose corresponding to 0·5 mg. of barium adenylpyrophosphate produced a small dilatation, followed by constriction of a dog's vessels, while equivalent doses of adenosine and muscle adenylic acid had no action at all.

Discussion.

The actions of the different drugs are summarized below.

Adrenaline. In dogs its chief action was constriction of the inflow. Its effect on the outflow varied. Its dilator action after ergotoxine was sometimes mostly on the inflow and sometimes on the outflow as well. In cats its chief action was dilatation of both inflow and outflow. This action was not abolished by ergotoxine. When constriction occurred, it was mostly due to an action on the inflow.

Histamine. Constricted both inflow and outflow in cats and dogs.

Acetylcholine. Small doses produced dilatation in cats and sometimes also in dogs. Larger doses produced constriction. In cats this occurred both on the inflow and outflow, but in dogs the action was mostly on the inflow.

Adenosine compounds. Small doses produced dilatation and larger doses constriction. In cats the constrictor effects of large doses were mostly due to an action on the inflow. Adenylpyrophosphate produced both effects much more actively than either adenosine or muscle adenylic acid.

Though we have recorded changes in the volume of blood in the lungs, our discussion has hitherto been confined to the consideration of the contribution of the different parts of the vascular system to the total resistance to the flow of blood. Most of the effects observed have been mainly due to vaso-motor changes in the inflow, but evidence has been presented which indicates that the action of histamine and adrenaline (see Figs. 2–6), and possibly also acetylcholine and adenosine, on the tone of the pulmonary veins may have an appreciable effect on the total resistance of the lungs to the flow of blood.

We must now consider what direct significance our results may have in regard to the effect of the drugs we have used on the blood content of the lungs in the intact animal. This is of importance from two points of view. In the first place, the lungs form one of the most important potential reservoirs for blood in the body [Magnus, 1930]. In the second place, the blood content of the capillaries is one of the factors which influence the length of time during which blood is exposed to the air in the alveoli. One of the factors controlling the blood content of the lungs is the hydro-

static pressure in their capillaries. This pressure must always be less than that in the pulmonary artery and greater than that in the left auricle. The mean of these two pressures is sometimes taken as an estimate of the capillary pressure [Magnus, 1930]. It is possible, however, that the stimulation of nerves or the application of drugs might produce changes in the capillary pressure, by acting selectively either on the arteries or the veins, so that the capillary pressure approached either that in the pulmonary artery or to that in the left auricle. The changes which we have recorded in the lung volume have been attributed to such a selective action, but these changes in volume were very small. It is just possible that vaso-motor actions might produce much larger changes under physiological conditions when the lungs are being normally ventilated, but it is improbable that this action is of much importance. The result shown in Fig. 3 may be taken as a demonstration that a drug may have an action on the outflow contrary to its action on the inflow, and that such an action can be detected by the methods we have used. This result was of doubtful interpretation, exceptional in nature and inconstant in appearance. In every other case in which an action on the pulmonary veins has been demonstrated or suspected this action has been in the same direction as the action which the drug had on the arteries in the same experiment. The pulmonary veins have, in fact, responded in such a way as to neutralize the changes in lung volume which would have occurred if the action of the drug had been solely on the inflow. There is thus no reason for supposing that the pulmonary veins play a part in the control of the blood content of the pulmonary circulation which is in any way comparable with the part which the hepatic veins have been found to play in the control of the blood content of the portal circulation.

Summary.

1. The lungs of dogs and cats were perfused with blood in such a way that either the perfusion pressure or the rate of flow could be maintained approximately constant. The blood content of the lungs was recorded by means of a plethysmograph.

2. The details of the effects seen are summarized above at the beginning of the discussion.

3. The plethysmographic record indicated that the most striking effects of adrenaline, histamine, acetylcholine and adenosine compounds were mainly due to an action on the pulmonary arteries, but evidence was obtained that changes in the tone of the pulmonary veins might also

have an appreciable effect on the total resistance of the pulmonary vessels to the flow of blood.

4. In almost every case where definite evidence was obtained that a drug was acting on the pulmonary veins, this action was such as to neutralize the change in lung volume which would have occurred if the drug had acted solely on the inflow.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

V. The adrenal cortex in pregnancy and lactation.

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Our finding, in recent experimental work [Andersen and Kennedy, 1932], of a significant difference between the adrenal weight of the rat at œstrus and at diœstrus has led us to investigate the adrenal changes in pregnancy. Without a preliminary knowledge of the changes in the non-pregnant animal it is impossible to have carefully standardized controls for the pregnant ones, and such knowledge has not been available to former workers. It was hoped that a series of pregnant rats of the same strain, kept on the same diet and in the same environment as that used in our former experiment, might yield valuable information.

Most recent text-books and reviews refer to an hypertrophy of the adrenals during pregnancy as an accepted fact. A careful analysis of the literature on the subject reveals that this belief is based on a rather small amount of data, and that the controls used in most of the experiments were inadequate, not only because the œstrus changes were unknown and disregarded, but because the possibility of an excessive hypertrophy of the cortex in pregnant animals that are infected over the non-pregnant ones as suggested by Donaldson [1924] in rats was not considered. Moreover, the changes described in the literature vary not only in different species, but in the same species as studied by different people. It is possible that not all mammalian adrenals respond similarly to pregnancy. The work on each species will therefore be considered separately.

Most of the work has been done on rodents, especially on guinea-pigs. The first report was by Guieyesse [1899, 1901], who gave a lucid description of various changes in the adrenals of his pregnant animals, the chief of which were the following: the cells of the middle third of the cortex were increased in size and contained larger vacuoles; the cells of the inner portion of the fascicularis contained a greater number of granules staining with iron hæmatoxylin; and those of the reticularis

contained a greater number of these siderophil granules as well as an increase in pigment granules. He found no mitoses and concluded that the increase was in the size, not in the number of cells. The zona glomerulosa was unchanged. The animals were killed between the tenth and fifty-second day of pregnancy. The chief criticisms of his work are that his controls were males, except for one immature female 2 months old, that no records of the number and ages of animals were given, and that no note was made as to infections in his animals. In view of the large size of the female adrenal as compared with the male, the first of these objections goes far to invalidate his findings as the specific effect of pregnancy. Marrassini [1906] reported on ten pregnant guinea-pigs with nine non-pregnant female and fourteen male controls, and described an increase of lipoids in the fascicularis but no change in the other zones. He found a greater variation in the weight of the adrenals in both non-pregnant and pregnant females than in the males. The three animals killed in early pregnancy resembled the non-pregnant females, while the three in late pregnancy and the four killed post-partum had larger adrenals. Inspection of his figures reveals the fact that six of the female non-pregnant animals were much lighter in weight than the pregnant ones. The remaining four are in the same weight range as the pregnant ones, if the probable weight of the uterine contents be considered, and the adrenals in these animals are in the same range of weight as the pregnant ones, rather less, if relative weight be considered. The male adrenals are smaller than those of the non-pregnant females according to his figures, although he describes them as being about the same. Ciulla [1910] also confirmed Guieyesse, but mentioned no controls and gave but few details. He also studied the proteolytic enzymes of cortical tissue and found about the same quantity in pregnant and non-pregnant animals, with wide variations. He quotes Costa [1906] as reporting a decrease in the lipolytic and proteolytic activity of the adrenal cortex during pregnancy.

Kolmer [1912] examined three pregnant, six lactating, and three non-pregnant guinea-pigs and confirmed the findings of Guieyesse with the addition of many mitoses at about the time of parturition. Kolde [1913] also confirmed the findings of Guieyesse in his study of four pregnant and two non-pregnant animals, one of the latter being very old. These observations are based on so few animals, in view of the range of variation of adrenal weight, that they may be disregarded.

Sternberg [1915] had a series of thirteen pregnant, eight post-partum, and five non-pregnant control guinea-pigs and confirmed Guieyesse as to hypertrophy of the fascicularis with increased lipoid

and without mitoses. He also described an hypertrophy of the glomerular zone. He includes a table of data which reveals the fact that actual weights are reported on only three of the five controls and that these three are small animals. Calculation of the weight of adrenal per kg. of body weight based on his data gives a result in the controls within the same range as in the pregnant animals. Sternberg's work can therefore be disregarded.

Verdozzi [1914, 1917] presented a series of twenty-four pregnant and twenty post-partum animals, and a description of the adrenal in the non-pregnant controls, the number of which was not given. He calculated the relative weight and made from his data a chart, in which there is a high peak at the end of the first half of gestation produced by the figures from two animals. The remaining figures for pregnant animals are low on the average but vary greatly. In eleven post-partum animals the young were left with the mother, and in nine they were removed. The figures for the former series are definitely higher than for the latter and appear significant.

The most recent work on guinea-pigs is that by Castaldi [1922] on a series of eleven pregnant animals with a number of non-pregnant controls, of various ages, of which six were older than 3 months. He confirmed Guieyesse. A study of the figures which he presents shows so great a variation that the difference is not significant, especially if the relative weight is calculated.

Other rodents that have been studied are the rat, mouse and rabbit. Herring [1920] found that the mean relative adrenal weight in nine pregnant rats was 0·22 g./kg. and in nine non-pregnant controls it was 0·20 g./kg., a difference that is not significant in view of the variation and the number of animals. Donaldson, in twenty-nine pregnant and lactating rats, with a mean body weight of 167 g., found a mean relative adrenal weight of 45·3 mg., while in twenty-nine non-pregnant controls with a mean body weight of 163 g. the mean adrenal weight was 44·7 mg., or practically the same. He pointed out a most interesting fact, that the infected pregnant animals had a much higher adrenal weight than the infected non-pregnant ones. Forty-seven pathological controls with a mean body weight of 174 g. had a mean adrenal weight of 47·9 mg., while fifty-one pathological pregnant and lactating rats with a mean body weight of 163 g. had a mean adrenal weight of 57·8 mg. In a later study Donaldson [1928] showed that the relative volumes of the medulla and cortex were the same in pregnant and non-pregnant animals.

In mice the problem is complicated by the presence, in this species, of

the X-zone lying between the fascicularis and medulla. Three workers, with an adequate number of animals, have described a decrease in size of the adrenals and the rapid disappearance of this zone during pregnancy [Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928]; Howard-Miller [1927] and Deanesly [1928] describe this phenomenon as the hastening of the normal age involution of this zone. All three workers investigated the presence of œstrus changes: adrenal hypertrophy was described by Masui and Tamura [1926], but was not found by Howard-Miller [1927] and Deanesly [1928].

Gottschau [1883] made the first observations on rabbits. In a series of twenty pregnant rabbits and nineteen non-pregnant females, he observed a slight decrease in size in the adrenals of the pregnant animals, while the adrenals of seven males were still smaller. Elliott and Tuckett [1906] give low figures in two pregnant and three non-pregnant females. Ciulla analysed the cortex of rabbits for fat and lecithine and found that the fat content was 10 p.c. by weight in the non-pregnant and 18 p.c. in the pregnant animals. Lecithine formed 50 p.c. of this in the non-pregnant and 72 p.c. in the pregnant. He gave but few details, and did not mention the number of animals, the age and weight, or the sex of the controls. Kolde [1913] found no difference between five pregnant and eleven non-pregnant females. Sternberg [1915], who studied eight pregnant and two lactating rabbits and five non-pregnant controls, reported changes similar to those in guinea-pigs. He gives complete data on only one control, but states that there is hypertrophy in the pregnant animals. This finding, in the face of the previous work and his inadequate controls, leaves one sceptical of his work, not only on rabbits, but also on guinea-pigs.

No observations on dogs have been found, but the figures given by Elliott and Tuckett on two pregnant and two lactating cats show about the same relative weight of the adrenals as in the non-pregnant females of his series.

The diagram given by Kolmer [1918], based on observations of eight pregnant, one lactating, sixteen non-pregnant female and twenty-five male moles, indicates adrenal enlargement during the breeding season (February to April) in both sexes. This enlargement was found in both pregnant and non-pregnant animals during the season of rut. The factor of seasonal variation in a hibernating animal complicated the picture.

In man, the most considerable series described in detail is that of Sambalino [1910], who reported a series of twenty-six cases of women dying in pregnancy or the puerperium. Many of these died after an

abortion or immediately post-partum of various causes. An abstract of his case histories reveals the following facts: he described marked hypertrophy in three cases, one of which died of pyelonephritis, and the others of puerperal infection; slight to moderate hypertrophy in sixteen cases, the cause of death being hæmorrhages in three, eclampsia in three, chronic nephritis in four, post-partum infection in two, pulmonary tuberculosis in one, pneumonia in two and pulmonary thrombosis following femoral thrombosis in one; adrenals of normal size in four cases, dying of acute nephritis, eclampsia, abdominal sarcoma and puerperal infection respectively; and very small adrenals in three cases dying of nephritis (two cases) and pernicious anæmia. He concluded that increased vacuolization in the zona fascicularis and increased pigment in the zona reticularis were changes due to pregnancy, and that adenomata were common in the cases of eclampsia and nephritis. However, according to the descriptions of individual cases, these are not always present. Moreover, the size of the adrenal was estimated from its appearance in the microscopic sections. There is no correlation between the degree of hypertrophy and the age of the patient or the number of pregnancies. The constant presence of any one change was not demonstrated. Chirié [1908] reported a series of twenty-eight autopsies on women dying during the terminal portion of pregnancy or soon after parturition. Of these, twelve died of eclampsia, four of nephritis, and the remainder died of various causes, chiefly infection. He observed hypertrophy of the cortex in most of the cases of eclampsia and nephritis with hypertrophy of the medulla in many of these cases. Hypertrophy of the cortex was found twice and hypertrophy of the medulla was not present in the remainder of the series. Cortical adenomata were about equally frequent in all types of cases. He concluded that the hypertrophy was a response to the intoxication resulting from renal changes. Minervini [1904] reported a case of a woman who died in the seventh month of pregnancy from eclampsia, and Gaifami [1907] reported four cases, in two of which the cause of death was streptococcus infection, and in the other two shock from hæmorrhage. Both observers described increased lipoid in the fascicularis and increased pigment in the reticularis. Kolde [1913] described the adrenals of five pregnant and five non-pregnant women. He found the zona fascicularis and zona reticularis large, and the glomerulosa small and with mitoses. There were no adenomata in his case of eclampsia.

Landau [1915], aware of Sternberg's work in the same laboratory, in a careful study of three hundred autopsies of adults of both sexes, was surprised to find no difference between adrenals of the cases dying in

pregnancy or during the puerperium (number of cases not given) and the remaining non-pregnant ones. Goormaghtigh [1922] added one case in which he found hypertrophy of the fascicularis with an increase of lipoid, and atrophy of the reticularis. The difficulties of evaluating autopsy material are manifest, especially in view of Donaldson's observations in rats of marked adrenal hypertrophy in infected animals, but not in uninfected ones, and Goormaghtigh's observations on the variability of the changes in infections following war wounds.

A critical review thus leads us to the conclusion that the evidence is against hypertrophy of the adrenal during pregnancy in the rat, mouse, rabbit and the cat. The hypertrophy found during the breeding season in the mole is found in both pregnant and non-pregnant animals as well as in males. There remain the conflicting findings of various observers of the guinea-pig. It has been shown that none of the work on the adrenals of pregnant guinea-pigs has been adequately controlled. It is possible that this animal differs in its reactions from other rodents, but the question must be left open until careful work has been done with non-pregnant female controls of the same breed, age and weight, with a study of possible changes associated with the œstrus cycle and with scrupulous elimination of infected animals. Notwithstanding the limitations of work on post-mortem material, the weight of evidence is against hypertrophy of the adrenals in pregnant women.

Technique.

The animals used were of the same strain and kept on the same diet and under the same conditions as those used in our studies on the adrenal in œstrus [Andersen and Kennedy, 1932]. They were all bred in our laboratory, so that the exact age was known. The majority were killed during their first pregnancy, although one of the uninfected and several of the infected ones had previously had litters. All but five were killed between the ages of 120 and 200 days, the remainder being a few weeks older. The cycles were followed by means of vaginal smears, and the animals were mated for one night during œstrus, so that the exact length of pregnancy was known. They were then killed in groups of four to six at the following periods of pregnancy and lactation: the tenth, eighteenth and twenty-first days of pregnancy; during parturition, with part of the litter born and part unborn; and on the first, seventh, fourteenth and twenty-first days of lactation. In all, the series comprises twenty pregnant and twenty-one lactating rats. Forty-one more animals

were used, but these were animals having some infection of the lungs or ears and were made to form a separate series. The experiments were carried on over the course of about 18 months as animals were available, and no seasonal variations were noted.

The animals were killed with chloroform, and the body weight both with and without the products of conception was noted. The animals were examined for infections, especially of the lungs and middle ears. The pituitary, thyroid, adrenals and thymus were then weighed in a closed weighing bottle and fixed in Zenker's solution. In some cases from each stage one adrenal was fixed in formalin. Both adrenals from eight animals were serially sectioned and stained with hæmatoxylin and eosin: of these, three were from animals killed on the eighteenth day of pregnancy, two on the first day post-partum, and three from the twenty-first day of lactation. Frozen sections stained for fat with Scharlach R were made of adrenals from animals of each phase. The series of animals studied in regard to œstrus and diœstrus and previously reported [Andersen and Kennedy, 1932] were used as controls.

The weights of the organs are calculated in terms of g. per kg. of total body weight and of net body weight. The total body weight is considered as the weight of the mother plus the uterus and its contents in the pregnant animals, or plus the weight of the litter in the lactating ones, conceiving of the young either unweaned or unborn as dependent on the mother for nourishment. The net body weight is the weight of the mother after the uterus and contents have been removed, or the weight of the lactating mother without regard to the litter. The results in regard to the adrenals will be reported here, and those in regard to the pituitary and thyroid will be reported in a subsequent paper.

In addition a group of four pseudo-pregnant rats, killed 1 week after mating with a vasectomized male, were included. Four rats killed after an anœstrus period of 3 weeks or more, and nine non-pregnant animals dying of spontaneous infections were also studied for comparison.

No animals were used which were known to be infected at the time of mating, and the high percentage of infections is surprising in view of this limitation, the adequate diet, ample light and cage space, and careful attention to cleanliness. All the infected rats had the same type of lung infection so commonly found in rats, in which smaller or larger chronic abscesses are scattered through the lung, with occasional solidification of one or more lobes. A few also had middle ear infections. A few of the animals in the infected series were about a year old, and were among the first to be examined. Unsuspected infections were found much more

commonly after the age of 6 months, and for the remainder of the experiment only young animals between 90 and 150 days of age at the time of mating were used. Since the degree of infection cannot be easily measured, and some rats having only a few abscesses had large adrenals, all animals having any evidence of infection whatsoever were eliminated from the normal series.

The calculation of the probable error was made from the formula

$$\text{P.E.} = \frac{2}{3}\sqrt{\frac{\Sigma x^2}{N}}.$$

Data.

The mean relative adrenal weight at various stages of pregnancy is given in Table I. It may be seen that in normal animals the relative adrenal

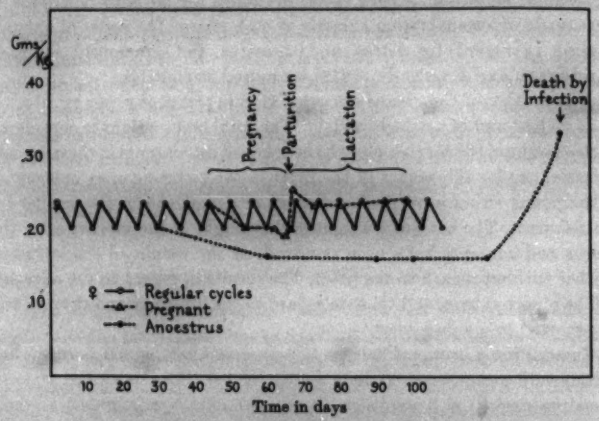

Fig. 1. The relative weight of the adrenal gland in pregnant, lactating, non-pregnant and anœstrus female rats. Body weight approximately 200 g.

weight is about the same in various stages of pregnancy. It is also quite uniform throughout lactation and is slightly heavier than in pregnancy. The largest figure, at 1 day post-partum, is due to a single large reading of 0·332 g./kg. in a rat in which no infection was discovered. This is more than 20 p.c. larger than any other rat of the entire series of normal pregnant or lactating animals, and is in the range of weight found at œstrus. In some cases the rat has a single œstrus period immediately

TABLE I. The mean weight of the adrenal glands in various stages of pregnancy.

Uninfected animals

Stage	No.	Body weight in g. Total with young	Body weight in g. Net without young	Adrenals mg.	Adrenals g./kg. total body weight	Adrenals g./kg. net body weight
10 days pregnant	4	204	203	41·6	0·205	0·205
18 „ „	7	234	208	41·2	0·177	0·200
21 „ „	4	255	199	37·9	0·150	0·192
Parturition	4	247	195	42·0	0·171	0·217
1 day post-partum	5	243	197	48·1	0·201	0·248
7 days post-partum	5	253	197	45·2	0·179	0·230
14 „ „	5	351	206	47·7	0·138	0·234
21 „ „	6	450	223	48·1	0·108	0·217

Infected animals

Stage	No.	Body weight in g. Total with young	Body weight in g. Net without young	Adrenals mg.	Adrenals g./kg. total body weight	Adrenals g./kg. net body weight
10 days pregnant	5	210	209	47·3	0·227	0·227
18 „ „	10	224	201	51·6	0·233	0·262
21 „ „	6	274	230	54·6	0·200	0·239
Parturition	—	—	—	—	—	—
1 day post-partum	11	259	213	53·2	0·208	0·253
7 days post-partum	—	—	—	—	—	—
14 „ „	—	—	—	—	—	—
21 „ „	9	373	211	48·2	0·129	0·229

post-partum and it was felt that this might be the case here, but unfortunately no vaginal smear was taken and this point could not be ascertained at the time that the large weight was discovered. The two animals of the same stage which were killed subsequently were carefully examined and had no œstrus changes in the uterus or in the vaginal smear, and, at the same time, had adrenals which corresponded in weight with those of the remaining rats of the series. The point, therefore, remains obscure.

The mean weights for the series of pregnant and for the series of non-pregnant animals are given in Table II, with the figures for non-pregnant œstrus, dioestrus, anœstrus animals of approximately the same weight for controls. A group of rats dying of spontaneous lung infections is added for comparison with the less severely infected pregnant and lactating animals. The weights for the pregnant animals correspond with those of the dioestrus series and are much less than those of œstrus. The weights for the lactating animals are higher, with a probable error that

TABLE II. The mean absolute and relative weight of the adrenal glands in pregnant and lactating rats as compared with non-pregnant female rats of various types.

		Uninfected animals		
			Adrenals	
Stage	No.	Net body weight g.	Actual weight mg.	g./kg. net body weight
Pregnancy	15	204 ± 18	40·4 ± 3·4	0·199 ± 0·010
Parturition	4	195 ± 11	42·0 ± 0·7	0·217 ± 0·013
Lactation	21	206 ± 18	47·3 ± 3·8	0·232 ± 0·021
Pseudo-pregnancy	4	203 ± 5	45·8 ± 3·3	0·227 ± 0·021
Œstrus	13	Over 180	49·2 ± 4·3	0·237 ± 0·016
Dioestrus	6	,,	42·0 ± 2·4	0·212 ± 0·006
Anœstrus	4	177 ± 7	29·0 ± 3·4	0·164 ± 0·018
Rats dying of infection	—	—	—	—

		Infected animals		
			Adrenals	
Stage	No.	Net body weight g.	Actual weight mg.	g./kg. net body weight
Pregnancy	21	211 ± 19	51·5 ± 6·1	0·247 ± 0·033
Parturition	—	—	—	—
Lactation	20	212 ± 22	50·9 ± 5·4	0·243 ± 0·024
Pseudo-pregnancy	—	—	—	—
Œstrus	—	—	—	—
Dioestrus	—	—	—	—
Anœstrus	—	—	—	—
Rats dying of infection	9	160 ± 26	69·0 ± 7·6	0·442 ± 0·046

is fairly significant. There is no correlation between the size or total weight of the litter and the size of the adrenal. The adrenal weights for the pseudo-pregnant animals correspond with those of the pregnant ones, as might be expected. The somewhat higher mean is the result of one large figure, while the other three correspond closely with the pregnant ones. It is obvious that hypertrophy of the adrenals in relation to reproductive phenomena in the rat is found in connection with œstrus and to some extent with lactation, but not with pregnancy.

The infected animals show much greater variation in the adrenal weight than the uninfected ones, with some increase in mean relative weight. The figures for the various phases of pregnancy and lactation cannot be interpreted to mean a greater or lesser adrenal sensitivity to infection, because the degree of infection varies and in some groups there are a greater number of severely infected animals. There is a rough correlation between the degree of infection and the degree of adrenal hypertrophy and only a few of the slightly infected animals have larger adrenals

than the normal ones. For example, of the five infected rats killed on the tenth day of pregnancy only one was severely infected, with consolidation of one lobe of the lung and many small abscesses in the other lobes, while the remaining four rats had only a few scattered small abscesses. The severely infected rat had an adrenal weighing 0·281 g./kg., while the heaviest of the others weighed 0·228 g./kg. Six of the eleven animals killed on the first day post-partum had quite severe infections, and most of these had large adrenals. The mean weight for this stage is consequently large. The rule does not hold exactly, however, for several of the severely infected animals in the series had small adrenals, and another factor or other factors must be present.

Hypertrophy of the adrenal in these spontaneous lung infections in the rat occurs also in non-pregnant animals. In a series of nine non-pregnant animals dying of such infections the mean actual adrenal weight was 69·0 mg. and the mean relative adrenal weight was 0·440 g./kg., a figure very much higher than that for the animals with milder infections.

A study of serial sections of the pregnant animals reveals no striking changes from the appearance of the dioestrus rat which has been previously described. There is a little more lipoid in the fascicularis in some of the lactating animals, as shown by the Scharlach R stain on frozen sections, but this is not so striking as to enable one to distinguish between the pregnant, lactating and dioestrus adrenals with any degree of consistency.

Discussion.

It is obvious from the data presented here that, in the normal rat, there is no adrenal hypertrophy during pregnancy. The weight and histological appearance correspond with that of non-pregnant dioestrus animals. This does not necessarily hold true for other species, such as the guinea-pig, in which the œstrus cycle is longer. In our series of four rats which had had no œstrus cycles for some weeks, the adrenals were smaller than those of either the dioestrus or pregnant animals. In the short cycle of the rat the post-œstrus or uterine phase of the cycle is so closely followed by the next œstrus that it is difficult to surely distinguish the post-œstrus from the dioestrus phase. It is quite possible that in an animal having a longer cycle, such as the guinea-pig, the adrenals continue to decrease in size after the post-œstrus period, assuming that œstrus hypertrophy in other animals occurs, which is as yet unproven.

An interesting point is brought out by a comparison of the actual weights and the relative weights in relation to the total and net body

weights as described above. In a study of the figures for the individual rats it is seen that the most constant figure is that of the relative weight per net body weight. The actual weights vary with the body weights. This greater uniformity and, therefore, greater significance of the relative weight per net body weight over the actual weight is also shown by the probable error, which is about 5·0 p.c. of the mean in the case of the relative weight and 8·6 p.c. of the mean in the case of the actual weight in pregnant animals. The relative weight in relation to the total body weight decreases as the products of conception increase, but varies greatly (Table I). For example, in one rat weighing 196 g. and 54 g. of uterus and contents, the actual adrenal weight is 39·5 mg., the relative weight per total body weight is therefore 0·158 g./kg., while the relative adrenal weight per net weight is 0·202 g./kg. or very near the mean for dicestrus and pregnancy. This means that the physiological burden on the adrenal is not sufficiently increased or altered by œstrus, lactation and infection. Whatever may be the function of the adrenal, it does not appear to be involved in the preparation of nutrient material for the embryos. The embryos themselves acquire relatively large adrenals at a very early stage.

That the adrenals of pregnant rats hypertrophy more readily in the presence of infection than those of non-pregnant animals as suggested by Donaldson [1924] is not clear, since the majority of the non-pregnant female rats of our colony with even a mild degree of infection have long periods of anœstrus. Those with slight infections have the very small adrenals found in the uninfected anœstrus rats, while those with more extensive processes have adrenals of varying sizes up to 0·090 g. A series of non-pregnant infected animals would, therefore, include adrenals both smaller than and as large as those in a series of pregnant infected rats, and the comparison is difficult. In our relatively short series the greater frequency of severe infections during lactation over the number found in the earlier stages of pregnancy may not be significant, but it is suggestive.

The question of a possible relationship between the corpus luteum and the adrenal cortex has interested many investigators from Mulon [1906] and Watrin [1914] on. In recent years several papers have appeared which deal with a difference in the survival period of pregnant and non-pregnant animals following adrenalectomy. A prolongation of the survival period in pregnant animals was first described by Stewart [1913] in cats; but the survival period in most of his animals was shorter than is considered usual in non-pregnant animals at the present time. A more marked prolongation of the survival period was reported by

Rogoff and Stewart [1927] in a series of seventeen pregnant dogs. The maximum survival period in seventy-four non-pregnant and male animals was 15 days (two dogs), while the majority died between the fourth and twelfth day. Of the seventeen pregnant animals five lived for 13 to 15 days and eight lived over 17 days, some of the latter surviving many weeks. They believed that this prolonged survival period was due to the presence of the corpus luteum, since they also obtained unusually long survival in two non-pregnant animals which were adrenalectomized towards the end of an œstrus period and therefore had corpora lutea. Corey [1928] repeated the experiment on cats and did not find a prolongation of the survival period in twelve pregnant animals as compared with three non-pregnant females and three males. The matter must therefore be left open, although the former report is more convincing, both because of the experience of the workers, and because of the greater significance of positive over negative findings in a question where operative technique is so important. The report by Carr [1931] of a prolonged survival period in pregnant rats may be disregarded, since non-pregnant rats may survive adrenalectomy indefinitely, presumably because of the presence of accessory fragments of cortex. If we accept the findings of Rogoff and Stewart, their interpretation must be left until further elucidation of the cortical function; the two hypotheses that appear possible are that the corpus luteum can act as a substitute for the adrenal cortex—a theory based largely on histological similarities; or that, for some other reason, less of the secretion of the adrenal cortex is necessary during pregnancy.

The present confirmation of Verdozzi's findings of hypertrophy of the adrenal during lactation does not greatly clarify the question of the relation between the corpus luteum and the adrenal. A large adrenal is found during œstrus when the corpora lutea are not active and during lactation when they are; a small adrenal is found during pregnancy and pseudo-pregnancy when the corpora lutea are active, and during dioestrus when they are not; and a still smaller one during anœstrus when neither corpora nor follicles are active. Yet the pregnant dogs of Rogoff and Stewart survived adrenalectomy longer than the non-pregnant ones. The resolution of these apparently incompatible phenomena into a logical hypothesis cannot be accomplished as yet, but it would seem probable that the relationship between the corpus luteum and the adrenal cortex is not a direct one.

It has not yet been proven that a hypertrophied adrenal is an overactive one, and this will doubtless not be settled until the function of

the cortex is known, since the hypertrophy of the adrenal is usually due to hypertrophy of the cortex. The conditions in which hypertrophy has been found are beriberi, starvation [McCarrison, 1921], some infections, œstrus or the mating season, and lactation; these are all occasions of especial strain on the organism. However, other types of strain are not accompanied by adrenal hypertrophy, and the common denominator in this series is not obvious.

Conclusions.

1. The mean actual weight of the adrenals in a series of fifteen pregnant rats is 40·4 ± 3·4 mg. The mean weight as expressed in terms of g. per kg. of the body weight of the mother minus the products of conception is 0·198 ± 0·010. There is but a small variation in the relative weights so calculated.

2. The corresponding figures for a group of four rats killed during parturition are 42·0 ± 0·7 mg. and 0·217 ± 0·013 g./kg. There is no definite change either just before, during, or just after parturition.

3. The mean actual weight of the adrenals in a series of twenty-one lactating rats is 47·3 ± 3·8 mg. The mean relative weight, calculated as described above, is 0·232 ± 0·021 g./kg. This is higher than in the pregnant animal, and appears to be a significant increase.

4. As compared with the non-pregnant controls described in a previous paper it is found that the relative and absolute weight of the adrenal in pregnant rats is the same as in non-pregnant rats examined during dioestrus, and is considerably less than the weight during œstrus. It is greater than the weight in animals killed after a long period of anœstrus. The adrenal weight in lactating animals is slightly less than that during œstrus.

5. A series of four pseudo-pregnant rats had adrenals resembling those of pregnant animals.

6. In pregnant and lactating rats which are found at autopsy to have lung infections the adrenal weight is much more variable than in normal animals. In moderate or severe infections it is 25–50 p.c. greater, and in the slight infections it is sometimes greater and sometimes not. The degree of enlargement corresponds roughly to the degree of infection.

7. The histological appearance of the adrenal in the pregnant animal resembles that in the non-pregnant dioestrus one. The adrenal of the lactating animal contains a little more lipoid in the fascicularis.

8. A critical survey of the literature reveals that the balance of evidence is against hypertrophy of the adrenals during pregnancy in the

rat, mouse, rabbit and cat. There is hypertrophy of the adrenals of both sexes during the season of rut in the mole, but this has not been shown to be characteristic of pregnant animals in particular. The evidence for such hypertrophy in the guinea-pig is not adequately controlled in any series and the truth of the matter is in doubt. The studies on human autopsy material yield conflicting results, although the most reliable work is against such hypertrophy.

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THE DIFFERENTIAL PHARMACOLOGY OF THE INFERIOR VENA CAVA OF SOME MAMMALS.

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In rabbits which have been killed by a blow on the head and have then been bled from the jugular veins, and in rabbits and cats killed and bled simultaneously by the humane killer, I have frequently found the inferior vena cava contracted caudal to the renal vein entries and apparently widely dilated between these and the liver. In anæsthetized animals, also, I have found that it is not always easy to insert a wide cannula into the posterior part of the vein because of its great contractility. I have, for instance, seen it shrink after being cut from a diameter of approximately 5 mm. to the thickness of a thread.

The reason for the contraction in cases of hæmorrhage may be, as suggested by the papers of Rous and Gilding [1929] and of Gilding [1929], that the blood supply to the muscles and skin is temporarily reduced while that to the upper part of the intestine, whence fresh fluid would naturally enter the body, is kept normal and, with it, that part of the inferior vena cava into which the portal vein empties. In cases of small hæmorrhage it is probable [Jarisch and Ludwig, 1927] that the vena cava would not be concerned, but only the portal system. That the extra contractility of the caudal portion of the vein may have another function besides this emergency one, however, is suggested by the normal lie of the venæ cavæ. This has already been pictured in the case of the rabbit and of the cat [Franklin, 1932 *a*], and the lie in the dog, sheep, and guinea-pig is similar in that, in the standing posture of the animal, the inferior vena cava ascends from the iliac veins to the renal veins and descends from these latter to the heart. The flow in the first part is against gravity, in the second part with it, and there is a tendency for veins which have to contend against gravity to be better equipped, *ceteris paribus*, with smooth muscle. There is also the point that the suprarenal veins discharge into the part of the vena cava between the renal veins and the liver, and it would be awkward for the body's economy if this portion responded more than the distal one to the secretion of the suprarenal medulla.

On all these counts it seemed worth while to compare the pharmacological reactions of the iliac portion (between the iliac and renal veins) and of the renal portion (between the renal veins and the liver) of the inferior vena cava. Waterman [1930] has shown that the vein[1] contracts with parasympathomimetic drugs, and I have also found contractions of minor degree with such reagents. To eliminate the possibility of parasympathetic stimuli being the cause of the contraction *in situ*, I took a ring which was so contracted and added atropine sulphate to the Ringer's solution in the beaker. There was no relaxation, so it seemed probable that the contraction was chiefly muscular or sympathetic in origin, and the latter interpretation is supported by the experimental results.

Veins from three species, namely, the rabbit, the cat and the dog, have been used, and have been tested by two techniques *in vitro*, and also by injection of adrenaline *in situ*.

In the experiments *in vitro* the reactions of vein rings to adrenaline 1:100,000 have been recorded. This concentration of the drug has been employed as likely [Franklin, 1932 *b*] to give a maximal effect, a conclusion justified by the fact that subsequent additions of 1 : 200 barium chloride usually caused no further contraction or, at best, only a small one. The recording was done by means of an optical recording apparatus, already described apart from small modifications [Franklin, 1930], and the movements were magnified 150 to 400 times, according to the size of the vein ring used.

Two different techniques were employed, as stated above. The first depended on the fact that the vein has a maximum distension point. This has already been pointed out in the case of the cat [Franklin, 1931], and was found to obtain likewise in the rabbit and in the dog (*q.v. infra*). This being so, it was assumed that, if the counterpoise in the case of any particular vein ring were so adjusted that it was the minimum weight which would give a maximum length of the vein between the hooks, this length corresponded to the maximum distension of the vein *in situ* and could be used as a zero. The only drawbacks to this were, first, that the measurements had to be made at room temperature, and on immersion in oxygenated Ringer's solution at body temperature there was a certain amount of contraction; and secondly, that this heat contraction affects also the connective tissue elements. If such a ring, however, is left for some time, it relaxes again, and one is probably not far wrong in taking the room temperature length as zero; at all events, the qualitative ratios

[1] Although not stated in the original paper, the part of the vein Waterman used was the abdominal, and not the thoracic.

are not affected, as comparison with the results of the second technique shows. The zero was recorded as soon as the vein ring was immersed in the Ringer's solution, and the difference between the zero and the highest reading produced by subsequent addition of adrenaline 1 : 100,000 was regarded as the maximum effect of the adrenaline for comparative purposes.

The second technique was suggested by Prof. J. A. Gunn, and depended on the observed fact that smooth muscle tends to relax right down if left for a long time in oxygenated Ringer's solution at body temperature. The length to which vein rings had relaxed after an hour in such an environment was, in these experiments, taken as zero, and the contraction thereafter produced by adrenaline 1 : 100,000 was recorded. At the maximum height of adrenaline contraction the lever holding the vein was fixed by tightening the side screw of the mirror spindle, and the length between the hooks was measured. The original length at zero was calculated from this, and thereafter the percentage diminution in circumference. The ratios between the contractions of the iliac and renal portions recorded by this technique agreed closely enough with the ratios found by the first technique on neighbouring vein rings. For instance, in the case of one dog's vein, they were respectively 2·5 and 2·2 : 1. The actual percentage contraction measured by the first technique was less than that measured by the second technique, but this was to be expected in view of the greater counterpoise used in the first method.

The experiments showed that the iliac portion of the vena cava of the cat, rabbit and dog was always more reactive to the given dose of adrenaline than the renal portion. The ratio was usually between 2–4 : 1, but in one case (a dog) it was even greater, 10 : 1. As these experiments recorded diminution in circumference, they corresponded with an even greater diminution in calibre of the vein *in situ*. The greatest decrease recorded was in the iliac portion of a cat's vein—88 p.c., as compared with 38 p.c. in the renal portion. Such a degree of contraction is rendered even more striking, when one remembers the heavy counterpoise, and it will not do to regard such a vein as a quiescent tube, playing only a passive part in the circulation of the blood. It may be noted, in addition, that on the whole the contraction of the iliac portions lasted longer than that of the renal portions; in no case was the reverse true, and in several the contraction of the renal portion was noticeably brief.

A final test of the comparative contractility of the two portions of the abdominal vena cava was made *in situ* on representatives of all three species, and it fully justified the conclusions already reached as a result of the experiments *in vitro*.

A buck rabbit, weighing 3020 g., was anæsthetized with ether. The superficial leg veins being too small in calibre, the abdomen was opened and a venous cannula inserted into a small tributary at the caudal end of the vena cava. The lower part of the animal was then immersed in a saline bath at body temperature. After it had been a while in this, the board was raised while the external diameter of the vein was measured with a pair of dividers at two fixed points in the iliac and renal portions. The calibres were recorded by pressing the points of the dividers in a sheet of paper. Then the board was again immersed for a while. After this 0·2 c.c. adrenaline 1:1000 diluted with 1·5 c.c. of warm saline was slowly injected through the cannula, and the calibres re-measured twice at intervals after the injection. The measurements so recorded were respectively 4·5 and 11·0 mm., 2·7 and 10·5 mm., and 3·1 and 10·5 mm., so the percentage diminutions in calibre of the two portions of vein were 40 and approximately 5. The animal was then killed with an overdose of ether, and a long upright tube connected with the venous cannula. When this tube was only partly filled with saline at body temperature, the vein had a calibre of 5·5 mm. in the iliac portion, and had not dilated beyond 11 mm. in its renal portion. Nor was there further dilatation on increasing the saline pressure to 160 cm. There was, therefore, a distension limit for the vein, and this was unaffected by removal of the mesentery. Also the calibre of the distended iliac portion was half that of the renal; this sort of ratio was found in the stretched veins of other rabbits. If the diminution in calibre under the action of adrenaline is calculated from the distended calibres, the percentage contractions become approximately 50 and 5.

A female cat, weighing 3600 g., was next anæsthetized with equal parts of ether and chloroform. The superficial leg veins were larger in this animal, and the venous cannula was therefore inserted into the one on the right side. The abdomen was opened, and the calibres of the iliac and renal portions measured before and after an injection of adrenaline diluted with saline, although in this case the injection was washed in with a further 1·5 c.c. of saline in view of the added distance to the vena cava. The figures for this animal were 4·3 and 7·2 mm. before the injection, and 2·5 and 7·0 mm. after it; subsequently the calibre of the iliac portion returned to 4·3 mm. The animal was then killed with an overdose of chloroform, the thoracic inferior vena cava cut open, and the inferior vena cava as a whole washed free from blood. The thoracic end was next clamped off, and the vein subjected to a pressure of warm saline up to 160 cm. After an increase in diameter with the initial rise of pressure to

calibres of 5·6 mm. and 7·6 mm. respectively, the iliac and renal portions alike showed an effective resistance to further distension. The contraction produced by adrenaline in the two portions was therefore approximately 42 and 3 p.c. of their calibre as calculated from the initial figures, or approximately 55 and 3 p.c. calculated from the maximum distension figures.

A dog, weighing 18 kg., and with a snout-buttock length of 99 cm., was anæsthetized with equal parts of ether and chloroform, and, after being used for another purpose, was killed with an overdose of chloroform. The abdominal vena cava was washed clear of blood, and subjected to an increase of internal pressure up to 160 cm. of warm saline, without increase in calibre after a few centimetres pressure. The calibre at two fixed points in the iliac and renal portions was measured at 18 cm. saline pressure before and after an injection of adrenaline, and the percentage diminution of calibre of the iliac portion was just less than twice that of the renal portion.

The fact that the vein soon reached a maximum distension point in all three animals is a justification of the first technique employed for the experiments *in vitro*.

The thoracic inferior vena cava was subjected to *in vitro* tests as a control, although histological examination of this vein from all three species has failed hitherto to reveal any appreciable number of smooth muscle fibres. Sections were fixed in Susa, and thereafter stained with picro-indigo-carmine and orcein. The essential structure of this vein in all three species is collagen and elastic tissue [Franklin, 1931, 1932 *a*], arranged in a special way. Occasionally, with the first technique, a ring from this part of the vein showed a small amount of contraction on immersion in the Ringer's solution at body temperature, but histological examination of a neighbouring ring failed to show more than a very few muscle fibres, quite insufficient in number to account for the contraction. Adrenaline also failed to suggest the presence of smooth muscle. Finally, no effect has ever been shown with the second technique. It may be concluded, from all these observations, that any contraction recorded with this portion of the vein by the use of the first technique was due to warming of the collagen or elastic tissues, or of both.

The relative sizes of circumference in the stretched iliac, renal, and thoracic portions of the inferior vena cava in typical examples of the three species were: rabbit, 10·4, 22·6 and 14·6 mm.; cat, 8·4, 13·4 and 12·0 mm.; dog, 23·2, 34 and 32·6 mm. When these figures are expressed as percentages of the iliac circumference, they become respectively 100,

217, 140; 100, 159, 143; and 100, 147, 141. The relative size of the iliac and thoracic portions seems fairly constant, therefore, in the three species, while the renal portion is comparatively much greater in the rabbit and slightly greater in the cat than it is in the dog. This feature is noticeable on inspection, but only this passing reference will be made to it here, as it demands a more extended investigation. It may also be stated here that the thoracic inferior vena cava of all three species tends to contract, on removal from the body, to a length slightly shorter than its expiratory length with accompanying increase in circumference. The above measurements for the thoracic portions are, therefore, possibly slightly greater than the measurements *in situ* in the expiratory phase of respiration, and markedly greater than those in the extreme inspiratory phase. The experiments on which these conclusions are based will be reported later on completion of the series.

To a considerable degree, then, in the rabbit, to a less but still noticeable degree in the cat, and to only a small degree in the dog, the portion of the inferior vena cava between the renal vein entries and the liver forms a dilatation *en route* to the heart. The reason for this dilatation and for the differences between the three species is at present so far a matter of conjecture that it is not worth while to make any suggestion; the existence of the dilatation and the much greater susceptibility of the more caudal portion of the vein to adrenaline are matters of fact.

Histological examination of the renal and iliac portions of the three species fails to show a sufficiently marked difference in the provision of circular smooth muscle to account for the much greater contractility of the iliac portions, and there are also certain species differences in structure. It may be that in this, as in certain other pharmacological findings, the solution must wait on biochemistry rather than on histology. At all events, it is wiser to defer any histological account until a much larger number of species has been examined. When that has been done, it may be more possible to correlate microscopical structure and physiological action, but in the meantime no useful suggestion can be made from this source.

Summary.

That portion of the inferior vena cava of the dog, cat and rabbit, which lies caudal to the renal vein entries is more reactive to adrenaline than is the portion between these vein entries and the liver. The thoracic inferior vena cava in these species did not give any reaction to adrenaline.

I wish to thank Prof. J. A. Gunn for his advice in connection with this investigation, Dr H. P. Gilding for the supply of some material, and Mr G. K. Elphick for occasional assistance. The expenses of the research have been defrayed by the generosity of the Rockefeller Foundation. The work forms part of a larger study of the venous return in mammals, but is here presented as a separate entity. A similar study of the inferior vena cava of primates is to be made, if possible, under the direction of Prof. J. F. Fulton in the Department of Physiology, Yale University School of Medicine.

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THE MEASUREMENT OF RED CELL VOLUME.

IV. Alterations in cell volume in hypotonic plasma.

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Ponder and Saslow [1931] showed by means of diffractometric measurements that the volumes assumed by red cells in hypotonic solutions of various salts are much less than those which would be expected on the supposition that the cells behave as "perfect osmometers," and accounted for the results by assuming that osmotically active substances are lost into the suspension media (hypotonic NaCl, KCl, and glucose). They attempted, also, to establish the same result for red cells suspended in hypotonic plasma, but not with conspicuous success, for the only method at their disposal for the measurement of the volumes in hypotonic plasma was the hæmatocrit method, which is not reliable [Ponder and Saslow, 1931]. The volume changes which the cells undergo in hypotonic plasma, however, are particularly important, for at least two reasons.

(1) The volumes attained in solutions of NaCl, KCl, and glucose, of various tonicities, were found from the diffractometric measurement of the radius of spherical forms. There is no doubt that the diffractometric measurements themselves are reliable [see Ponder, 1932], but the objection can always be raised that the same kind of forces which render the cells spherical when between a slide and cover glass alter their semi-permeability and render them liable to lose osmotically active substances.

(2) It can also be objected that changes of *p*H may occur when red cells are suspended in NaCl, and particularly in glucose. More specifically, this type of objection is related to the recent conclusions of Jacobs and Parpart [1932], who account for many of the phenomena of osmotic hæmolysis in terms of changes in the base-binding power of hæmoglobin. Jacobs and Parpart give their conclusion in the form of an equation,

$$\frac{W_1}{W_0} = \frac{2R+1-F_1}{2R+1-F_0}, \qquad \text{.....(1)}$$

in which R represents the ratio of base in the cells to the cell hæmoglobin, F_1 and F_2 the amounts of base bound by unit quantity of hæmoglobin under two conditions corresponding to the presence of quantities of water W_1 and W_2 in the cell (*e.g.* two different pH's), and where the concentration of salt C in the suspension medium is constant: if F is constant, however, and C is varied,

$$\frac{W_1}{W_2} = \frac{O_2}{O_1}, \qquad \text{.....(2)}$$

i.e. the cell behaves as a "perfect osmometer." They find that expression (1) satisfactorily describes the results of those experiments in which F_1 and F_2 are varied by changing the pH, the temperature, or the degree of oxygenation of the hæmolytic system, and conclude that, although the existence of factors "other than purely osmotic ones" is by no means excluded, such factors are probably of secondary importance and better disregarded until further calculations based on better data have been made.

It is therefore important to examine the behaviour of erythrocytes in hypotonic plasma rather than in hypotonic saline, for in the latter medium the abnormally small volumes observed might conceivably be due to the operation of such factors as Jacobs and Parpart discuss (particularly pH changes), while in the former medium such factors are constant and expression (2), which treats the cells as perfect osmometers, ought to apply. A difficulty arises, however, when we come to look for a method of measuring the volumes directly. The colorimetric method [Ponder and Saslow, 1930] cannot be used for very hypotonic solutions without becoming exceedingly complicated; the hæmatocrit method is not reliable, and the diffractometric method is not applicable to cells in plasma, which are discoidal. We have accordingly used three methods of quite a different character, all based on very simple principles.

I. Methods.

(*a*) *Red cell density.*

The principle of this method is that the volume of water taken in by the red cells, and therefore their increase in volume in a hypotonic solution, can be found from the values for their density before and after such swelling. The percentage increase in volume being known, the amount of "free water" (erroneously regarded as such) contained in the cells can be calculated on the assumption that water exchange is the only factor concerned in the swelling and that the cells behave as perfect

osmometers. The extent to which this figure for the "free water" departs from the true value (about 95 p.c. of the total water) enables us to judge the extent to which the cells depart from being "perfect osmometers" by losing osmotically active substances into the surrounding medium [see Ponder and Saslow, 1931].

Rabbit blood is obtained from the carotids, potassium oxalate being used as an anti-coagulant. The percentage volume of the cells present is found by the hæmatocrit, the tubes being spun at 4000 r.p.m. for 15 min. The density of the cells, suspended in their own plasma, is found by the method described elsewhere by one of us [MacLeod, 1932]. This determination alone requires about 15 c.c. of blood.

About 25 c.c. of the whole blood is set aside, and plasma is obtained from the remainder. The tonicity of this is taken as unity, thereby avoiding difficulties attendant on the conversion of the tonicity into an equivalent solution of NaCl. The plasma is then diluted with distilled water to give a hypotonic plasma, *e.g.* if a final tonicity of about 0·7 is required, 70 c.c. of plasma is diluted with 30 c.c. of water. It is convenient to make the total volume of the diluted plasma 100 c.c. This volume is then added to 20 c.c. of the whole blood and the mixture allowed to stand for a few minutes: all operations should be carried out at constant temperature, which, in the case of these experiments, was 27°. The 120 c.c. of mixed blood and plasma is now transferred to centrifuge tubes, the cells packed, and their new density found as before. This new density is always less than the initial value, for the cells have taken in water.

The tonicity of the 100 c.c. of diluted plasma is, of course, increased by the addition of the plasma contained in the 20 c.c. of added blood, but the new tonicity of the medium can easily be calculated. The assumption is now made that the cells, originally in equilibrium with plasma at a tonicity of unity, take in water so as to come into equilibrium with the diluted plasma surrounding them; the quantity of water which 100 c.c. of cells must gain in order to do so is then given by

$$x = \frac{100\,(d_1 - d_2)}{d_2 - 1}, \qquad \text{.....(3)}$$

where d_1 is the initial density and d_2 the density after the swelling has occurred. The volume thus increases from 100 to $100 + x$, and so the percentage increase in volume can be found.

A very small correction is now applied to the figure for the tonicity of the diluted plasma, for a quantity of water has been lost from it and has passed into the cells. Call the corrected tonicity T and the quantity of

water transferred to the volume of cells present in the particular experiment x'. Then the apparent quantity of water W_1 in the cells is

$$W_1 = Tx'/(1-T). \qquad \text{.....(4)}$$

Finally, the actual quantity of water present in the cells, W_2, is found by weighing a quantity of packed cells before and after drying at 60° [see MacLeod, 1932]. The fraction of the total water which is "free" is $R = W_1/W_2$.

The only significant sources of error in this method are those associated with the density determinations. Even if we allow an error of $-0{\cdot}001$ in d_1 and an error of $+0{\cdot}001$ in d_2, the error introduced into the value of x only amounts to about 3 c.c. in 20 c.c., i.e. a volume increase of 20 p.c. becomes one of 17 p.c. This leads to an under-estimation of R, in a typical experiment, of about 0·10, but, since such errors in the density determinations are unlikely to occur, and since the difference between the behaviour of the cells and that of perfect osmometers is far greater than could be accounted for by such an error in the evaluation of R, the method is adequate for our purpose.

(*b*) *Hæmoglobin determinations.*

The principle of this method is that the hæmoglobin content of unit volume of packed cells is reduced if the cells take in water, and that the amount of water so taken in can be calculated from the extent of such dilution.

Blood is obtained as in the first method and the percentage volume of the cells determined. The cells of about 5 c.c. of blood are packed in a rapid centrifuge, the supernatant fluid carefully removed, and the percentage volume of the packed cells found by hæmatocrit. Usually the percentage volume is more than 95 p.c. With the greatest care, 1 c.c. of the packed cells is removed and added to 1000 c.c. of 1 p.c. HCl. This constitutes the "standard."

About 25 c.c. of plasma is obtained, and its tonicity denoted by 1·0, as before. This is suitably diluted with water so as to give 25 c.c. of hypotonic plasma, which is then added to 5 c.c. of whole blood. After a few minutes the mixture is centrifuged and the supernatant plasma removed. The percentage volume of the packed cells is again determined: 1 c.c. of the packed cells is then added to 1000 c.c. of 1 p.c. HCl. This constitutes the "unknown."

The standard and the unknown are matched against each other in a colorimeter with a Newcomer eyepiece, and the quantity of added water

calculated from the degree of hæmoglobin dilution in the unknown. Corrections of an obvious nature are made for the increase in the tonicity of the hypotonic plasma by the addition of the plasma contained in the added blood, and for differences between the percentage volume of the packed cells obtained from normal plasma and hypotonic plasma respectively. The remainder of the calculations are the same as in the first method.

This method has about the same degree of accuracy as the density method, the principal errors being those associated with the estimation of the percentage volume of the packed cells and with the transference of the 1 c.c. of packed cells from normal and hypotonic plasma respectively. With care the latter can be made quite small, although it is always difficult to transfer such viscous material with great accuracy.

(*c*) *Total water.*

Just as the total quantity of water contained per unit volume can be determined for cells taken from normal plasma, so can it be determined for cells which have been immersed in hypotonic plasma. The methods used for finding the total water present are those described by MacLeod [1932], the water being driven off at 60° from cells from normal and hypotonic plasma respectively, until constant weight is reached in each case. The cells from hypotonic plasma, of course, always contain a greater percentage of water. The calculations are made along obvious lines, similar to those employed in the two foregoing methods.

II. Results.

(*a*) *Cell density.*

The following results are typical of those obtained.

T	d_1	d_2	W_1	W_2	R
0·646	1·0979	1·0820	1·78	3·21	0·55
0·645	1·0993	1·0822	2·88	4·63	0·62
0·734	1·0969	1·0856	2·76	4·25	0·65
0·846	1·0994	1·0918	3·00	4·16	0·72

The values of R lie between 0·55 and 0·72, as in experiments in hypotonic saline and hypotonic plasma (diffractometric and hæmatocrit determinations, Ponder and Saslow [1931], and as in Warburg and Winge's hæmatocrit determinations in hypotonic plasma). It is clear that in this latter medium, despite the constancy of pH, etc., the cells do not behave as "perfect osmometers," but lose osmotically active substances into the surrounding fluid, as has, indeed, been shown by the direct analyses of Kerr [1929].

(*b*) *Hæmoglobin determinations.*

This method gives substantially the same results as the foregoing. For example,

T	x	x'	W_1	W_2	R
0·646	23·0	0·437	0·08	1·24	0·64
0·732	16·1	0·322	0·88	1·26	0·69
0·829	11·1	0·200	0·97	1·70	0·57
0·863	7·8	0·164	1·03	1·95	0·53

Again R varies between 0·53 and 0·69, and we are led to the same conclusion as before.

(*c*) *Total water.*

The results of the total water determinations agree remarkably closely with those found by the density method. This can be seen from the following table, which gives the percentage increase in volume in media of various tonicities, as obtained by the two methods.

	x	
T	method (*a*)	method (*c*)
0·646	19·3	19·1
0·645	21·0	22·5
0·734	15·5	15·2
0·846	8·5	8·6

These results are of particular significance because the method used is so simple and direct. Consider, for example, the case where $T = 0{\cdot}646$, and where the cells in isotonic plasma contain 66 p.c. of water. If these were brought into osmotic equilibrium with hypotonic plasma at $T = 0{\cdot}646$, their volume would increase from 100 to 136 p.c.; if an exchange of water alone were responsible for the volume increase, we would expect the value of x to be 36 p.c. The fact that it is always about half this value constitutes the strongest argument against the behaviour of the cell as a "perfect osmometer."

III. Discussion.

Taking these results together with those published in previous papers, we can draw the following conclusions.

1. Every method by which volume measurements have been made, whether directly or indirectly, leads to the same general conclusion, viz. that the rabbit red cell swells less in hypotonic media than it would if water exchange alone were involved. The evidence on this point is very strong (colorimetric determinations, Ponder and Saslow [1930]; diffractometric determinations, Ponder and Saslow [1931]; hæmatocrit

determinations, Ponder and Saslow [1931], Warburg and Winge [1928]; and the determinations by the three methods described in this paper). Taken together with the results of Kerr [1929], these results force one to the conclusion that the cells lose osmotically active substances into the surrounding hypotonic fluid.

2. There is no essential difference between the results obtained in hypotonic NaCl and KCl and those obtained in hypotonic plasma. This disposes of the possible objection that the abnormally small amount of swelling is primarily due to *p*H changes occurring in the former media.

3. It follows from the preceding that expression (2), as given by Jacobs and Parpart, is incorrect, for it states that the swelling in hypotonic solutions can be accounted for by water exchange alone if *p*H, temperature, degree of oxygenation, and other factors affecting the base-binding power of hæmoglobin are kept constant. But direct measurements of cell volume show that this is not the case, even in hypotonic plasma in which *p*H, temperature, and the other factors are kept constant as required. Further, if expression (2) is incorrect, expression (1) must be incorrect also, for it is based on the same assumptions. There is no doubt, of course, that changes in the base-binding power of hæmoglobin affect the osmotic pressure of the cell interior and therefore the figure for the tonicity which produces hæmolysis, and that they operate in the direction which Jacobs and Parpart indicate; it is impossible, however, to express their effects in quantitative form until we are able to express, with quantitative exactness, the effect of the loss of osmotically active substances from the cell[1].

4. In spite of its relatively indirect nature, the diffractometric method of measuring red cell volume seems to yield essentially the same results as do other and more direct methods. This conclusion is of considerable importance from a purely technical point of view.

[1] Jacobs and Parpart claim that expression (1) is quite satisfactory for describing results which they have obtained in experiments in which *p*H and other factors were varied. But the experimental verification of their equation rests, not on direct volume measurements, but on an assumption which may well turn out to be incorrect, viz. that the cells hæmolyse at exactly the same critical volume, irrespective of the *p*H, etc., of the hypotonic medium in which they are placed. The agreement between their experimental and calculated results, moreover, is only to "the same order of magnitude," which is scarcely sufficient.

Summary.

The quantity of water taken up by red cells when immersed in plasma of varying degrees of hypotonicity is found by three methods: (*a*) by determining the decrease in density of the cells consequent on this intake of water, (*b*) by measuring the diminution in hæmoglobin content of the swollen cells, and (*c*) by determining the quantity of water taken in by drying the cells and weighing. It is shown that the amount of water taken up by the cells as they swell is much less than that which would be expected if the cells were "perfect osmometers," *i.e.* if they took in water without losing osmotically active substances into the surrounding hypotonic medium. This is the same kind of result as has already been obtained for cells immersed in hypotonic NaCl, KCl, and glucose.

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THE INVESTIGATION OF THE TOXIC EFFECTS OF LARGE AMOUNTS OF SUGAR IN THE BLOOD.

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MANY experiments have been recorded in the past in which toxic effects are ascribed to the introduction of large amounts of sugar in the blood. On the other hand, observations have also been repeatedly made in which no such symptoms could be detected as a result of this procedure.

Among the former may be mentioned Harley [1893] who describes in dogs tremors, acetonuria, exaggerated respiratory movements, convulsions and coma; and Kossa [1899, 1911] who noted in fowls muscular weakness and inco-ordination. Allen [1913] who reviews the whole subject adds deaths from lowered resistance in guinea-pigs and rabbits, and anorexia and ataxia in dogs and cats; while Williams and Swett [1922] and Stoddart [1924] describe rigor and fever when unbuffered solutions are used.

None of these or any other toxic symptoms were observed by Kleiner and Metzer [1913, 1914], Albritton [1924], Boyd, Hines and Leese [1925], La Barre and de Cespédès [1931], in dogs, nor by Lamy and Mayer [1904] in dogs and rabbits, nor by Jüttemann [1930] in guinea-pigs, nor by Rigler and Uhrich [1923], Thalhimer, Raine, Perry and Buttlers [1926], Lennox and Bellinger [1927] in man.

In few of these experiments were high concentrations of blood sugar recorded, and Harley [1893] with a reading of 676 mg. per 100 c.c. and Kleiner and Metzer [1913, 1914] with readings of 720 mg. in normal and 1100 mg. per 100 c.c. in depancreatized dogs are the observers recording the greatest sugar concentration. In order to re-investigate this matter, large quantities of glucose were injected intravenously in normal rabbits with the object of observing any acute, toxic or nervous phenomena as the result of high concentration of sugar in the blood.

Description of experiments.

Varying amounts of glucose solution 10 and 15 p.c. in sterile distilled water were injected intravenously into the veins of one ear of a rabbit. Samples of blood were taken from the veins of the other ear. Normal rabbits with large ear veins were used, no anæsthetic was administered, and the time of injection was on an average 20 minutes. The ears were shaved and a fine "Record" needle attached to a syringe (10 or 20 c.c.) inserted in a vein, and held in position by an assistant. The animals were placed in a box which fitted them comfortably, for convenience of handling during the experiment.

The amounts of sugar in the blood were estimated by Hagedorn and Jensen's method. Certain modifications had to be carried out, as 2 c.c. of potassium ferricyanide are completely reduced when the percentage of sugar is above 385 mg./100 c.c. In the first six experiments, 0·1 c.c. of blood was taken and further amounts of 2 c.c. of potassium ferricyanide were added as the solution became colourless on boiling; and several blank determinations were made according to whether 2, 4, 6 or 8 c.c. were used. In the last three experiments, in order to lessen the volume of fluid at the final titration, smaller amounts of blood were taken when it was suspected that the sugar content was high. In the seventh experiment duplicate estimations were carried out, using 0·025 and 0·05 c.c. of blood respectively, and the results agreed fairly accurately, 1044 and 1060 mg./100 c.c.

Results of experiments.

(1) *Blood sugar curves.*

The first readings prior to injections showed a normal value for blood sugar. No readings were taken during injection. Following the injection high blood-sugar values were found. In the earlier experiments less amounts of glucose were injected and the readings were 600 to 900 mg./100 c.c., in the later experiments when more glucose was injected values of 1300 to 1900 mg. were obtained. The blood sugar fell rapidly, so that in most cases the level was normal again in 3 hours. In the last experiment when the blood-sugar level of 1900 mg./100 c.c. was reached, the level had only fallen to 236 mg. in 4 hours. In one experiment the sugar in the blood from the animal's heart was found to be 1380 mg./100 c.c., a reading similar to that which would have been expected from blood in the ear.

TABLE I.

Exp.	Animals, weight, date	Glucose injected: Strength of solutions p.c.	Glucose injected: In c.c. of solution	Glucose injected: In g.	Duration of injection min.	Time of samples after injection min.	Blood sugar values in mg./100 c.c.	Comments
1	Female 2 kg. 12. viii. 31*	10	80	8	30–45	Before	129	Urine sugar + + present
						3	627	
						5	480	
						20	466	
2	Male 2 kg. 13. viii. 31	10	120	12	—	4	953	Control blood sugar 122. Urine sugar + +
						5	876	
						10	762	
						40	561	
						85	242	
						140	171	
3	Female 13. viii. 31	10	45	4·5	—	Before	125	
						5	595	
						8	563	
						10	530	
						40	421	
						90	134	
						165	130	
4	7. i. 32	10	110	11	20	Before	64	
						1	887	
						10	684	
5	2 kg. 11. i. 32	15	90	13·5	10	Before	140	
						1	1051	
						2	892	
						12	660	
						35	638	
6	19. i. 32	15	150	22·5	40	Before	131	
						2	1335	
						20	911	
						55	728	
						125	565	
7	2. ii. 32	15	90	13·5	40	Before	89	
						1	1052	
						170	300	
8	9. ii. 32	15	180	27	20	Before	91	
						1	1616	
						25	988	
						180	132	
9	16. ii. 32	15	200	30	20	Before	107	
						1	1902	
						25	> 1100*	
						235	236	

* Tube broke during experiment.

(2) *Effect on rabbit.*

Large quantities of urine were passed which reduced Benedict's solution; the rabbits ran about the laboratory immediately after the injection, and there was no evidence of inco-ordination of movement, weakness of muscles, or coma. The hair of the rabbits remained good, and no trophic lesions were noted, except occasionally when a hæmatoma had occurred at the seat of injection, or sugar solution had passed into the subcutaneous tissue. No chills or rigors were noted; the rabbits did not always feed well for a few days following the injection. One animal became pregnant during the course of the experiments and later gave birth to a litter of seven, four of which survived and are now healthy rabbits. This was an unintentional result, since it was not known that the rabbit was pregnant. The two rabbits on which the last six experiments were carried out were alive and well 6 months after the last injection.

One of the rabbits was killed 7 months after the last injection and a post-mortem examination made. No abnormality was seen on macroscopic examination of heart, brain, pancreas, kidneys, suprarenals, liver, spleen, testes and blood vessels. The lens of the eye was crystal clear. Microscopic examination of the pancreas, spleen, aorta, suprarenals, kidneys, heart muscle and testes showed no abnormality. The liver also appeared normal, but only very small amounts of glycogen were demonstrated histologically.

Discussions.

In these experiments it was intended to observe any acute toxic effects of high concentrations of sugar in the blood. With the exception of one animal that died (and which was found to have a dilated heart) none were seen. No toxic after-effects became apparent. Higher blood-sugar concentrations were achieved than have been previously described in the literature. The blood-sugar value rapidly fell and the longest period of hyperglycæmia (1900 falling to 230 mg./100 c.c.) was 4 hours.

Summary.

1. Experiments are described in which large quantities of glucose were injected into the blood stream and very high readings of sugar concentration are recorded.

2. No pathological effects could be attributed to the raised blood sugar.

The experiments were carried out at the Physiological Department, St Bartholomew's Hospital Medical College. I wish to thank Prof. Hartridge and Dr Gordon Reeves for their assistance in preparation of this paper, and I am indebted to Dr George Graham and Dr Harrison for helpful suggestions concerning the experiments.

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THE RATE OF BLOOD FLOW AND GASEOUS METABOLISM OF THE UTERUS DURING PREGNANCY.

By J. BARCROFT, W. HERKEL and S. HILL.

(*From the Physiological Laboratory, Cambridge.*)

Barcroft and Rothschild [1932] found that the uterine vessels of the rabbit contained, towards the end of pregnancy, large amounts of blood, sometimes as much as 30 c.c. An answer to the question whether this blood is being stored or is in active circulation demands measurements of the quantity of blood passing through the uterus per minute, and any further discussion requires some knowledge of the extent to which that blood is utilized by the uterus. The present paper describes an enquiry into these problems, but deals only with the period between the fourteenth and twenty-eighth days of intra-uterine life—that is through the period of growth of the fœtuses from the time when they begin to be weighable. The last two days of pregnancy present some rather baffling problems, which must be the subject of further enquiry.

The volume of blood which traverses the uterus.

The literature appears to contain no data with regard to the quantity of blood which flows through the uterus per minute.

Dissections.

The blood vessels in the rabbit, which lead from the generative tract, have been described in a former paper [Barcroft and Rothschild, 1932]. After testing several procedures we settled down to the following as being one which enabled us to collect all the blood from the uterus (though not from the ovaries, Fallopian tubes or vagina) without serious prejudice to the blood-pressure. The rabbit was given dial (about 0·6 c.c. per kg.). This hypnotic though in many ways satisfactory is uncertain as regards dosage; if the dose indicated proved insufficient, some ether was given as well. (Another rabbit to be used as a donor was similarly anæsthetized and a cannula inserted into its carotid artery.)

The dissections were carried out in the following order:

(1) A cannula was tied in the right external jugular vein for the reception of blood from the donor.

(2) A cannula was tied in the left carotid for the blood-pressure tracing.

(3) A small incision was made in the right side through which the right ovary was withdrawn and tied off together with the tissue in which it was embedded. The ligature included not only the ovary but also the Fallopian tube and incidentally the vascular connections of the genital tract *via* the right ovarian vessels. The incision was then sewn up.

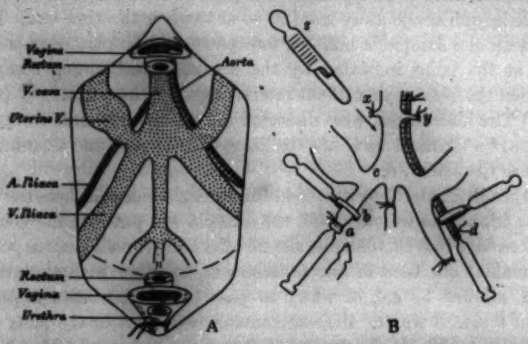

Fig. 1. A, General disposition of vessels as seen after removal of bladder, rectum and vagina. B, dissection showing the vessels tied and clipped, and the cannula inserted.

(4) The left ovary, Fallopian tube and vessels were treated similarly.

(5) A median incision was made over the bladder. After tying the ureters and the necessary vessels on the surfaces of the bladder and vagina a ligature was passed round the urethra, the rectum and the lower part of the vagina; this was firmly tied. Another ligature was passed and tied round the rectum and the upper portion of the vagina just below its junction with the uterus. The bladder, vagina and rectum were then cut out.

Through the window so made the veins appear (after clearing up the overlying tissue) as in Fig. 1 A. Our first efforts were in the direction of using one side of the uterus only, by inserting a cannula for the collection of the blood at *a*, Fig. 1 B, and, when we wished to collect the uterine blood, removing the clip from *b* to *c*. This procedure did not

succeed because the artery and the vein at *c* are often so nearly inseparable that the artery was interfered with by the clip, if not actually included in it. We therefore settled down to the following dissection which was carried out in the order here given. The coccygeal artery and vein were tied with a single ligature. The small veins *x* and *y* were tied and cut; according to the exigencies of the case they were tied either with one ligature or with two. The arteries corresponding were usually, but not always, treated in the same way. The vena cava was separated from the aorta and a loop of thread passed loosely round the latter. The aorta could then be drawn a little to one side without stopping the blood flow. Thus it was possible completely to explore the vena cava and dissect all tissue away from it, so that when the time came for the collection of a sample, a bull-dog forceps *z* could be placed on the vena cava at the point indicated by the arrow. This bull-dog completely occluded the vena cava without interfering with the aorta.

(6) The iliac vessels were dissected out on the left side, from below Poupart's ligament upwards, the iliac vein was tied and a cannula was tied into the artery, *d*.

(7) Similar dissections were made on the right side, but here the artery and its tributaries were tied and the cannula was put into the vein at *a*. It is necessary that this cannula should offer as little resistance as possible. The smallest one used in our experiments, together with the attached tubing, allowed 30 c.c. of water to pass per minute with a pressure head of 6 cm. of water. Measurements showed that in the later stages of pregnancy the blood-pressure at *c* was about 6 cm. of blood, *i.e.* if an open tube were attached to the cannula the open end had to be raised 6 cm. above the point *a* in order to prevent the blood from flowing out. It appeared therefore that if the blood flow was 30 c.c. a minute the cannula would introduce approximately the correct resistance. If the blood flow were less than this the veins might be expected to empty themselves a little before a steady flow through the cannula took place—for this reason in collecting a sample a little blood was usually allowed to go to waste between the moment of putting the bull-dog on the vena cava and collecting the blood for analysis. When the dissections were complete heparine was injected—75 mg. of the powder supplied by Messrs Hynson, Westcott and Dunning of Baltimore in 10 c.c. of Ringer was the usual amount. In some of our latter experiments we used the fluid preparation of Jacobi which we found to be equally satisfactory. The registration of arterial pressure was then begun and blood passed into the jugular vein from the carotid of the donor.

The collection of blood samples.

Everything was now ready for the collection of blood samples, and this was done as rapidly as possible; the times at which the samples were collected were registered approximately on the blood-pressure tracing, and the actual intervals during which the venous samples were collected were measured with a stop-watch.

The usual routine was to withdraw an arterial sample of about 3 c.c., put it under paraffin and then collect three venous samples in rapid succession.

The venous samples were obtained as follows:

(1) The clip at *b* was removed and the rubber tubing attached to the cannula was held with its open end at a level sufficiently high to prevent the blood from coming out.

(2) The clip *z* was put on the vena cava and the end of the rubber tubing lowered to a level just below that of the vein. Now all the blood from the uterus and none, or a negligible quantity, from elsewhere was flowing down the tubing. After a little blood had escaped, the open end of the rubber tubing was placed under the surface of some liquid paraffin in a separating funnel and blood was collected for, say, half a minute; at once a second similar sample was collected and then a third. The times were not half a minute in all cases; in the rabbits at early stages of pregnancy, and in which the blood flow was slow, "minute samples" were taken and in some experiments the time interval was $\frac{3}{4}$ of a minute. Our aim was to obtain some sort of equality in the quantities of blood withdrawn. It is questionable whether our policy was the best. It would have been simpler and possibly no worse to have adhered to half-minute samples throughout.

It may be desirable here to touch upon our greatest source of solicitude, that of being justified in the belief that the rates of flow which we measured really represented those in the animal. The points for consideration were:

(1) That between the actual capillaries of the uterus and the cannula there is a large venous bed in which a considerable quantity of blood could accumulate or from which it could drain. Behind our measurement lies the assumption that the quantity of blood in the venous bed does not alter to any significant extent whilst we are collecting our samples. The assumption so made is probably correct when the quantity of blood collected in the first half-minute is the same as that collected in the second, for it is unlikely that the venous reservoir would fill or drain at

a constant rate over so long a time. In the main, we had been successful in securing a pretty constant rate of flow over the first two periods of collection.

(2) The withdrawal of blood naturally tends to lower the general arterial pressure and slow the blood flow through the uterus. In our earliest experiments we endeavoured to replace the blood withdrawn by defibrinated blood injected into the jugular vein. This technique was disappointing. Possibly we should have been more successful had we tried the injection of heparine blood.

The method which we adopted was as follows. At the end of our dissection the blood-pressure was usually about 90 mm. The rabbit was then supplied with blood from the donor which raised the pressure somewhat at first. After the pressure reached 110–120 mm. there was little or no further rise even though more blood was run in; it seemed probable therefore that this extra quantity of blood could be withdrawn without any considerable fall of blood-pressure.

In practice we found that when we were taking our venous samples, the first two samples caused very little alteration in general arterial pressure but that the drop became more marked during the withdrawal of the third sample, and the blood flow correspondingly slower.

In the following discussion we adopted the plan of accepting the figures for the first minute of collection. This decision was of course arbitrary. Frankly it must be admitted that such a course does not exclude considerable errors. Our principal justification is that we tried several methods for the calculation of our results and they all gave the same general picture. It is only this general picture which we would stress.

A rather attractive method was that of accepting the rates of blood flow for the second half-minute only, on the ground that those for the first half-minute were more likely to be vitiated by alterations in the venous reservoir, whilst those in the third half-minute were more likely to be vitiated by alterations in the general arterial pressure. Having accepted the rates of blood flow for the second half-minute, the gas analyses for the third half-minute could be combined with them on the ground that the blood collected in the third half-minute was that which actually traversed the capillaries during the second. This procedure seemed complicated and suggestive of a claim to accuracy which we do not wish to make.

Results.

Fig. 2 shows the rate of blood flow from the vein plotted against the period of embryonic life which has been reached. The maximum rate of flow is about 30 c.c. per minute.

The comparison of the results shown in Fig. 2 with those of Fig. 4 in a recent paper by Barcroft and Rothschild [1932] yields some points of interest.

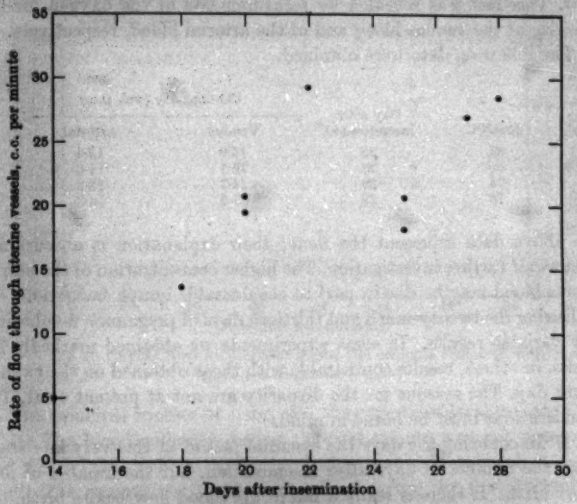

Fig. 2. Rate of flow of blood through the uterus from the fourteenth to the twenty-eighth day of pregnancy.

(1) The amount of blood which traverses the uterus per minute is of the same order as the volume of blood in the organ at any one time. In this the uterus appears to occupy a position intermediate between say the kidney in which the amount of blood in the organ at a given time is small relative to the amount which traverses it per minute, and the spleen where, at times, there is a large volume of blood that is normally almost stagnant. This relatively slow flow is doubtless due to the great development of veins in the broad ligament.

(2) The values obtained for this blood flow confirm those obtained for the blood content in showing that the quantitative vascular changes take place in advance of the embryonic growth. Thus as early as the fourteenth day the blood flow is quite rapid, 5 c.c. per minute, whilst by the eighteenth or nineteenth day it has reached 15 c.c. per minute, about half the maximum; yet on the eighteenth day the embryos are perhaps only a twentieth of their ultimate weight.

(3) We may emphasize the fact that the quantity of blood which emerges from the uterus appears to be less than that which is supplied to it. This fact was revealed by measurements of the oxygen (or CO) capacity of the venous blood and of the arterial blood, respectively.

The following data were obtained.

Rabbit	Day after insemination	CO-capacity (vol. p.c.)	
		Venous	Arterial
64	22	15·0	13·4
70	28	16·7	14·0
74	29	14·7	13·3
76	29	17·5	15·3

The above data represent the facts; their explanation is obscure and must await further investigation. The higher concentration of the uterine venous blood may be due in part to considerable lymph formation.

During the twenty-ninth and thirtieth days of pregnancy we obtained very variable results. In some experiments we obtained markedly low results, in others, results comparable with those obtained on the twenty-eighth day. The reasons for the disparity are not at present clear. Two considerations must be borne in mind.

(1) In counting the days the essential factor, at this very late stage, is not the number of days after insemination, but the number of days before birth. If there is really a fall in the blood flow before birth, this for example might appear on the twenty-ninth day if the young were to be born on the thirtieth and not till the thirtieth day if birth was to take place on the thirty-first. Hence variable results for the twenty-ninth day might be obtained.

(2) It is possible that shortly before birth the blood flow waxes and wanes. We made some observations of the pressure at the junction of the iliac and uterine veins during birth itself. The pressure rose with each peristalsis, fluctuating between 3 and 13 cm. of blood. At what stage this fluctuation first appears we do not know.

The oxygen metabolism of the pregnant uterus.

Fig. 3 shows the quantity of oxygen used by the uterus according to our very crude calculations. The figures are those for the first minute of our observations. They are obtained by subtracting the oxygen content of 1 c.c. of venous blood drawn in the first minute from that of 1 c.c. of arterial blood and multiplying the result by the average rate of flow during that period.

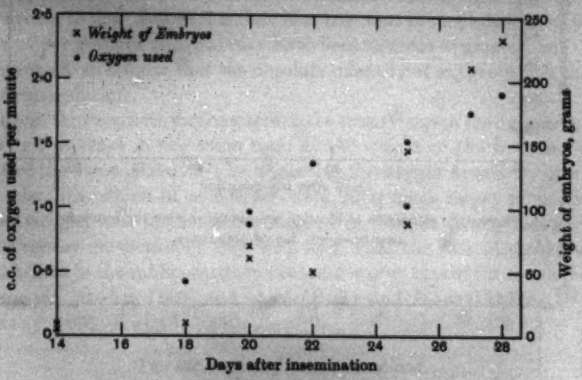

Fig. 3. Oxygen used by pregnant uterus and contents compared with aggregate weight of fœtuses.

The principal sources of error, and they are considerable, are:

(1) The blood collected during the first minute was not necessarily that which traversed the capillaries during that time interval.

(2) The uncertainty of the blood flow during the first half-minute owing to the large venous reservoir.

(3) The fact that no allowance has been made for the concentration of the blood.

Yet great as are these sources of error any variation which they cause would not alter the large features of the picture:

(1) That the oxygen usage up to about the eighteenth day is trivial, being under 0·1 c.c. per minute.

(2) That between the eighteenth and twenty-second days it increases rapidly, up to about 1·35 c.c. per minute.

(3) That between the eighteenth and twentieth days it undergoes sudden increase, attaining values of about 1 c.c. per minute.

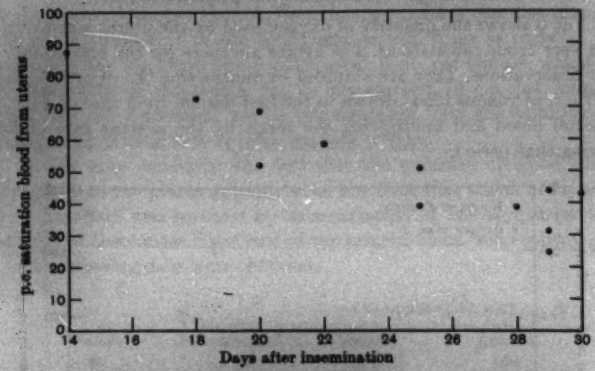

Fig. 4. Percentage saturation of blood in uterine veins from the fourteenth to the twenty-eighth day of pregnancy.

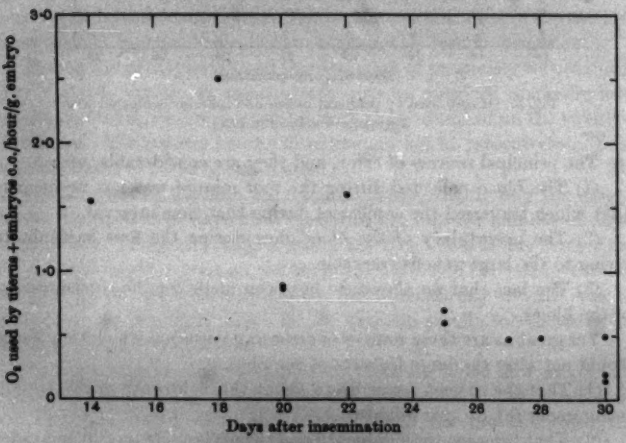

Fig. 5. Ordinate: oxygen consumption of uterine contents divided by weight of embryos. Abscissa: days after insemination.

(4) That after the twenty-second day there is a gradual increase, the ultimate figure being about 2 c.c. per minute.

As the increase in the size of the uterus between the eighteenth and twentieth days is not very remarkable, we may conclude that after the eighteenth day the metabolism of the uterus itself forms a negligible factor in the total which is almost entirely attributable to the embryos[1]. The significance then of the great rise in oxygen metabolism between the eighteenth and twentieth days is due to the sudden growth of embryonic tissue. Before this date it is true that the metabolism of the embryo per gram of material may have been as great or greater, but the embryos are so minute that the absolute quantity of oxygen which they use is insignificant.

From the twentieth day onwards to the twenty-eighth the oxygen used does not increase in the same ratio as the weight of the fœtuses. In spite of the error introduced by neglecting the oxygen uptake by uterus and placenta, which of course accounts for a much larger proportion of the oxygen used in the earlier stages, Fig. 5 shows clearly that there is a decrease in metabolic rate with age. Thus the rate of embryonic metabolism in the rabbit varies in the same way as that of the developing guinea-pig [Bohr, 1900] and chick [Bohr and Hasselbalch, 1900; Murray, 1926; Hanan, 1928].

The saturation of the venous blood.

The facts: (1) that the vascular conditions are established largely before the embryos are of appreciable size, and (2) that the embryos in the last fortnight grow out of all proportion to the vascular bed, explain the fact that starting from over 90 p.c. saturation the oxygen in the venous blood steadily falls till at the end of pregnancy saturation drops to between 45 and 25 p.c. (Fig. 4).

If as a first approximation one views the circulation in the uterus as consisting of bloods of very different oxygen tensions reaching the organ (*i.e.* the bloods in the uterine and umbilical arteries) and bloods approximately in equilibrium leaving it (*i.e.* the bloods in the uterine and umbilical veins), we arrive at a conclusion which is in line with those of previous authors who have made direct analyses of the blood in the umbilical vessels. Cohnstein and Zuntz [1884], Huggett [1927] and Haselhorst and Stromberger [1930] showed that the tension of oxygen in the blood which supplies the fœtuses is very low, 20–30 mm. Hg.

[1] More recent observations by one of us (Herkel) suggest that the metabolism of the uterus itself during pregnancy is greater than we had supposed.

When we get as low as 20 mm. we are getting to a critical point on the dissociation curve. Between 60 and 20 mm. pressure large changes in saturation take place with small changes in pressure, but any reduction of the blood below the point at which we have arrived means a drop in pressure at an ever-increasing rate. If the two blood streams which leave the uterus are in approximate equilibrium it is clear that intra-uterine conditions have practically reached an impasse. Further growth of the embryos would demand more oxygen, but that oxygen could not be supplied from the available supply except at the expense of a grave decline in the already small pressure at which the gas is supplied to the embryos. This pressure is already of the same order as, or below, that in the alveolar air at the top of Everest.

The rôle of the uterine veins.

We are now in a better position to discuss, though perhaps not to answer, the question which formed the original *motif* of the present paper. To what extent may the uterine vessels be regarded as a blood depot?

The discussion really concerns only the great development of veins.

Is this vast bed necessary for the transport of the quantity of blood which passes along it? The answer must surely be "no." For this answer three reasons may be given:

(1) The following argument may be applied: (*a*) Approximately the same quantity of oxygen must pass from the placenta to the fœtuses as from the maternal blood to the placenta. (*b*) The maternal blood being more than half reduced in the process, it may be assumed that at least as much blood passes along the umbilical veins as the uterine veins: yet, (*c*) The umbilical veins are delicate structures and, as compared with the uterine veins in the rabbit, are of almost negligible size.

(2) Each uterine vein at the end of pregnancy appears larger than the iliac vein into which it flows—yet there seems to be little difference in size between the portion of the iliac vein above the uterine vein and that below it.

(3) Veins no larger in other parts of the body carry much greater quantities of blood.

When we speak of a depot, however, there is the suggestion that the blood is stored for some specific purpose. For what purpose could blood be stored in the uterine vessels, to be liberated suddenly at parturition? The obvious suggestion is for the supply of mammary tissue much

hypertrophied during the active process of lactation. We do not know the quantity of blood in the mammary glands, but a few rather crude measurements suggest that they contain much less than 30 c.c. This, however, must form the subject of future enquiry.

Another useful function of a store of blood would be insurance in case of hæmorrhage during parturition. Meanwhile it is scarcely possible to accept the idea of the great veins in the broad ligament being a blood depot without considering some possible alternative functions. Of these the most probable appears to be protection against a too high venous pressure. This may be considered in three connections:

(1) The copious anastomoses which exist go far to prevent pressure on any one vein from producing stasis in the corresponding placenta.

(2) In several experiments we have been much impressed with the tendency of even moderate pressure on the uterine vein to produce intra-uterine hæmorrhage. On two occasions, not in this but in a former series of experiments, when there was some slight bleeding in veins near the main uterine vein and in which pressure was used very cautiously to stop that bleeding, considerable hæmorrhage was found in the uterus of the same side and not of the opposite side.

(3) It has already been noted that at birth the pressure at the mouth of the uterine vein oscillates within wide limits. It is possible that with the blood being driven from the smaller vessels of the uterus in a more or less rhythmic manner, this large venous bed may act as a sort of shock absorber, filling and emptying according to the rate of flow from the uterus itself and insuring against so great a venous pressure as to produce copious hæmorrhage.

Summary and conclusions.

1. The blood becomes appreciably concentrated in its passage through the uterus.

2. The blood flow through the uterus increases in volume in proportion to the increase in the vascular bed and anticipates the growth of fœtal tissue.

3. The blood flow in the rabbit's uterus attains a maximum of about 30 c.c. per minute.

4. The oxygen used by the pregnant uterus and its contents is less than 0·1 c.c. per minute up to the eighteenth day; within the next two days it undergoes a tenfold increase, corresponding to fœtal growth. After that time the oxygen used increases much more slowly and at a rate proportionately less than the growth of the embryos.

5. The oxygen saturation of the venous blood which is from 80 to 90 p.c. on the fourteenth day falls to 25 to 45 p.c. by the end of pregnancy.

6. The function of the extensive venous reservoir in the broad ligament is discussed (*a*) in relation to its possible function as a blood depot, and (*b*) as an insurance against stasis or intra-uterine hæmorrhage caused by local pressure.

Our thanks are due to the Royal Society for a grant which defrayed a part of the expense of the above research.

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THE COMPONENTS OF THE RETINAL ACTION POTENTIAL IN MAMMALS AND THEIR RELATION TO THE DISCHARGE IN THE OPTIC NERVE.

By RAGNAR GRANIT (*Helsingfors*)[1].

(*From the Physiology Laboratory, Oxford.*)

Part I. Isolation of components in the retinal action potential of the dark-adapted decerebrate preparation.

Our knowledge of the retinal action currents, discovered by the Swedish physiologist Holmgren [1882] in 1865, has proceeded hand in hand with the development in electrophysiology in general. The history of this striking progress in electrical recording is briefly summarized in the literature relating to retinal action currents. Since Gotch [1903], working in this laboratory, with the aid of the sufficiently fast capillary electrometer, obtained the first curves embodying all the features of the process, and since v. Brücke and Garten [1907] and Piper [1911] in extensive series with the string galvanometer had shown the responses to light to be fundamentally alike for various vertebrate eyes, the main features of the retinal action currents have been common knowledge to all physiologists. Valve amplification was used at an early stage for the investigation of retinal action potentials by Chaffee, Bovie and Hampson [1923]. Unfortunately they used excised opened bulbs, although the method was particularly well suited for the study of intact animals, a feat attempted as early as 1876 by Dewar and McKendrick [Dewar, 1876]. With their slow Thomson galvanometer the latter authors even succeeded in obtaining responses from the human eye, but it remained for Hartline [1925] to prove by systematic comparisons with the string galvanometer that the deflections obtained from intact animals were identical with those given by the bulbs. Hartline also recorded some fairly good retinal action currents from the human eye.

The retinal action currents have generally been held to be composite effects. In view of the complex structure of the retina and the equally complex appearance of the potential change accompanying stimulation by light, interference phenomena between potentials differing in sign,

[1] Fellow of the Rockefeller Foundation.

strength and time relations would certainly offer a reasonable explanation of the effect in terms of simpler components. Several such solutions have been propounded [see *e.g.* Kohlrausch's review, 1931], the best known being those of Einthoven and Jolly [1908] and of Piper [1911]. Evidently it is theoretically possible to resolve a complex curve in an infinite number of ways. And, though a many-sided experimental experience may make certain solutions more probable than others, yet a final decision can only be reached when the composite curve has been split into components by biological means. Such an attempt forms the subject of this paper.

The work has been based on the assumption that an organ like the retina where cells have become differentiated for specific purposes may show selective sensitivity or selective resistance to certain agents. It then becomes of paramount importance to find a preparation sufficiently stable and yet sufficiently sensitive to serve for the analysis. Frogs were tried but soon discarded in favour of the Sherrington decerebrate cat preparation [cf. Hartline, 1925]. This proved very satisfactory, provided that no operations were carried out around the bulb. In the best animals the first positive deflection, the *b*-wave, remained constant within 4–5 p.c. for several hours. The secondary rise varied more. Some thirty animals were used and the number of photographed responses approached 800.

Animal technique.

Cats were decerebrated under deep anæsthesia by a "backward" section generally carried down to the base of the skull. A dilated pupil was thus obtained. In a few cases atropine had to be given to immobilize the iris. This did not seem to influence the action potentials. The modified decerebration technique, described by Bazzett and Penfield [1922] and used by Hartline [1925] in order to prevent interference with anastomotic connections at the *basis cranii*, was not found (in ten preparations) to possess any advantages over the ordinary clean section. The nictitating membrane was removed and the lids tied apart. The carotid of the side to be recorded from was temporarily occluded during the operation.

The preparation was placed in a shielded and earthed box, the inside of which was painted black. The head of the animal was fixed in a specially constructed clamp and adjusted with one eye towards the opening of a tube leading to the stimulus and entering the box. This was placed on a heated table of the type used in this laboratory: 2 to 3 hours were allowed to elapse before any records were taken. The box had then been

closed for 1½ hours or more, except for the moment when the corneal electrode was applied. This was done about half an hour before the first records were taken. Faint light was used during this procedure.

Apparatus.

Contact with the cornea and the decerebration wound was made through cotton wicks leading to U-tubes filled with Ringer solution into which dipped silver-silverchloride wires. The leads were taken to the input of a directly coupled amplifier containing two valves coupled against one another in what is generally known as a "push-pull" arrangement. A small permanent magnet string galvanometer (Edelmann's small model) was placed in a bridge between the anode circuits and balanced to zero by potentiometers in the grid circuits. This coupling scheme, for which I am indebted to Dr H. K. Hartline of the Johnson Foundation, University of Pennsylvania, gives a circuit practically free from drift. A different type of directly coupled amplifier was used by Chaffee, Bovie and Hampson [1923].

The valves were 41 M.X.P. Cossor with indirectly heated filament, high mutual conductance, and low amplification factor. The actual amplification is only about five times. The use of valves was dictated, not so much by any necessity of amplifying the retinal currents, as by the advantage of having a potentially worked device such as the grid of a valve. This renders the resistance in animal and electrode negligible. Valves also make it possible to use the fairly insensitive permanent magnet string galvanometer. Further advantages are the cheapness of this galvanometer and the fact that the magnet and the case of the string remain at a practically constant temperature during the experiment. There is thus no need for continuous adjustment of string tension followed by calibration. As a matter of fact the calibration current was turned on only once or twice an hour, depending on the nature of the work. A disadvantage of amplification in this particular type of work is that not only action potentials derived from the retina but also those caused by movements in the eye muscles, lids, etc., are amplified. However, it can be seen from the records published below that good preparations are fairly stable.

The string was generally kept aperiodic or just periodic with a rising time not exceeding 20σ. The fastest *b*-waves recorded rose in about 50σ to their maximal value. The deflection to 1 mv. was rarely over 40 mm. through animal and amplifier. A platinum string 2 micra thick and with a resistance of 3100Ω was used throughout the work.

Since the permanent magnet string galvanometer has not been used very much as a measuring instrument on account of its low sensitivity, it may be useful to mention the disadvantages connected with this apparatus. The adjustments for string tension and focus are crude. The microscope and the magnet are not well balanced on the small upright to which they are attached. Hence the apparatus is very sensitive to mechanical disturbances. The string cannot be precisely centred in the magnetic field and the proportionality range is low. The latter fact is of little concern when the string can be kept slack. The range of proportionality was tested and found to be satisfactory within the limits of deflection used in this work, where not only was the string fairly slack but the optical magnification was considerable (approximately 600 times).

The stimulus was supplied by a Primus projection lamp (1000 c.p.) with reflector. The light beam was led through a system of lenses and screens and focussed to a narrow opening in a diaphragm. An adjacent minute slit let part of the light through a series of prisms to the camera. A Compur photographic shutter at the focussing point interrupted stimulus- and signal-beam simultaneously. The light reached the box through a system of concentric tubes of which the innermost possessed a ground glass disc with an area of 1661 sq. mm. on which an image was formed.

Though several intensities were used the typical effects are well illustrated by the highest intensity, 14 millilambert, and a 100 times lower intensity. The high intensity will be referred to in the text as I, other intensities as fractions of I. The intensity was varied by means of Wratten filters and was measured by a Lummer-Brodhun contrast photometer and a lamp standardized by the National Physical Laboratory. These were obtained when the apparatus had been used for nearly 5 months, so that the intensities given do not take into account the ageing of the lamp.

The cat's retina.

Some eyes were fixed in "Susa" and stained in Heidenhain's iron-hæmatoxylin. In accordance with older observations [cf. *e.g.* Chiewitz, 1889] the retina was found to contain very few cones. Also the convergence of receptors towards ganglion cells was found to be considerable, between 30–60 cells in the external nuclear layer per ganglion cell, in sections from various parts of the retina. In the human periphery the corresponding figures vary between 20–80, the latter value in the outermost parts of the retina [Chiewitz].

The normal action potential.

Some typical specimens are shown in Fig. 1. *A*, *B* and *D* are at the highest intensity, *C* is at I/100, *E* is at I/10. *A* is taken at a slower speed with the stimulus lasting 11 sec. and 5 sec. cut out from the film. The secondary rise is the largest noted in these experiments. A suitable terminology for designating the various phases of the retinal action potential was suggested by Einthoven and Jolly [1908]. Thus the initial fast negativity is called *a*, the first positive rise *b*, the slow secondary rise *c*. The off-effect (Gotch's term) was lettered *a'* by Einthoven and Jolly. Below, *d* is used [Day, 1915].

The three high-intensity curves show typical variations in the relative size of the waves. *B*, again, is typical of the large majority of the high-intensity responses. The *a*-wave is only present with high intensities and with large areas. The same holds for the off-effect or *d*-wave, which is not always present and is hardly ever more than a retardation in the falling part of the *c*-wave [cf. similar observations by Piper, 1905, 1911; Kohlrausch, 1918]. The very simple character of the response at low intensities should be noted; likewise the fact that occasionally, as in *D*, the base line may be re-attained for a moment after the *b*-wave at high intensities. The *a*-wave is not an abnormal phenomenon due to excessively high intensities of stimulation. It is a perfectly normal phenomenon and can, for instance, be seen in the human response curves published by Hartline [1925]. Though 14 ml. as a maximal intensity is not very high for the light-adapted human eye, it is a strong stimulus for a dark-adapted nocturnal animal with a wide-open pupil.

The effect of area.

This question will be treated in greater detail in Part II, where some measurements of the latent period of the action potential will be published. Here it is only necessary to emphasize the fact that a diminution in area affects the action potential just as a diminution in intensity [cf. Kohlrausch's review, 1931]. Thus the response for small areas of about 1–2° of visual angle differs from that for areas over about 5° in lacking secondary rise, the *a*-wave and the *d*-wave. They resemble the low-intensity curve *C* of Fig. 1.

Fröhlich [1928] and Fröhlich, Hirschberg and Monjé [1928] contend that the ordinary complicated responses depend upon the fact that the leads are not localized to the point of stimulation. Kohlrausch [1918, 1931] has convincingly shown that this hypothesis is untenable.

It may be added that the alleged proofs published by Fröhlich, Hirschberg and Monjé refer to experiments in which the area stimulated was not only kept just in front of the electrode but was also diminished in order to obtain strict localization. Inasmuch as the responses then became simplified, this result might just as well be ascribed to the diminution in area as to the fact that the lead was at the point of stimulation. In my experiments the corresponding lead was taken from the decerebration

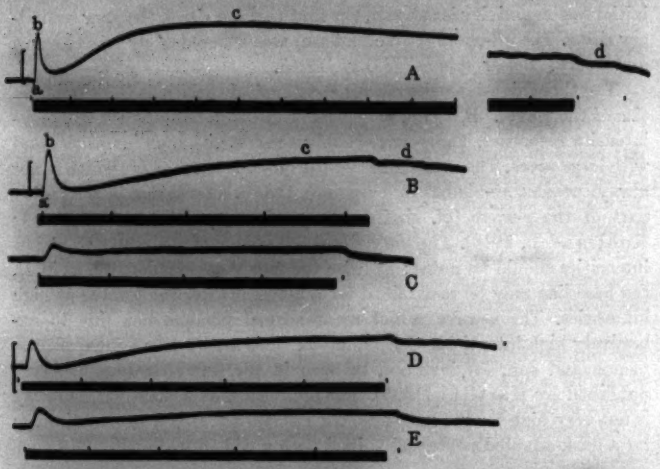

Fig. 1. *A*, intensity I; 5 sec. cut out. *B*, intensity I. *C*, intensity I/100. Standard area at 70 mm. in this and all following records except *D* and *E*, which are taken with the same area at 370 mm. at respectively I, and I/10. The potential developed in *D* and *E* is unusually low even for this retinal area, though normal in appearance. The deflection to ½ mv. is marked on one of each of the records of a series with the same animal, in this and in all illustrations following. Time is marked in all records in 0·5 sec. In addition time is marked by the shadow of the Rayleigh wheel giving 100 and 20σ when visible in reproductions of original curves.

wound on the skull and yet the responses became simplified by merely diminishing the area. No localization of the electrode is therefore necessary. The results to be described below put Fröhlich's theory definitely out of court. But the manner of leading off naturally is of some importance, particularly with a large excised eye as used by Fröhlich in some cases (Cephalopod), where probably his explanation holds.

Removal of c-wave.

The *c*-wave varies a great deal in height from animal to animal. It is extremely slow as is well shown by Fig. 1, curve *A*. It generally reaches its maximum in about 2 sec. or slightly less, and may last for several seconds after stimulation.

In order to remove it ether was chosen as a differentiating agent and was found to be very satisfactory. It was administered by way of inhalation. Fig. 2 (Plate I) shows three ether experiments in which the high-intensity stimulus was employed. In the upper pair of curves it can be seen that narcotization has removed the rising component of the *c*-wave in 14 min., and that the fast components are unaltered. The string remains at a practically steady low potential. In the next pair of records, of which the upper one again is the control, the picture after 14 min. of narcotization is similar, but the base line has shifted a little towards the end of stimulation. It is important to note that in this record the off-effect is enhanced by narcotization. This, in fact, is very often found. The third pair of records shows a case where 9 min. of anæsthesia has left the fast components unchanged, but greatly diminished the secondary rise. Whereas in some animals the rise may be completely removed by narcotization without any measurable effect on the fast initial components, there are certainly cases where part of the secondary rise is left at a stage of anæsthesia when a diminution of the *b*-wave is already noticeable.

It can be shown that the remaining part of the *c*-wave, in cases where something does remain, is not identical with the component that has been removed. The procedure is to analyse the effect of narcotization at two intensities. In Fig. 3 the upper pair of responses shows the control curve and the potential elicited by the stimulus I after 29 min. of narcotization. The lower pair shows the corresponding curves at I/100. The ether effect on the responses is very different. Whereas the *c*-wave in the high-intensity record has diminished by 54 p.c., it is only diminished by about 8 p.c. at the low intensity. High-intensity records were taken before (the one published) and 2 min. after the low-intensity record, and showed that the lack of effect with the lower intensity did not depend upon insufficient narcotization or recovery from the anæsthesia. The explanation is clearly that the rising component, which is such a marked feature of the high-intensity response, is minimal at the 100 times lower intensity. Other experiments showed it to be absent or below the instrumental threshold at still lower intensities. The low-intensity curves were unchanged at a stage of anæsthesia showing high-intensity responses

affected as in Fig. 2. In this manner, then, it is possible to determine the time course and magnitude of the slow secondary rise, and, in fact, to isolate it by subtraction.

It is evident that the purely descriptive term, *c*-wave, has a double sense in terms of components. The *c*-wave is not homogeneous at high intensities. With certain animals nearly the whole of the secondary positive potential can be removed with ether at high intensities. It is then not identical with the corresponding component at low intensities which is unaffected. The component that is so easily removed by narcoti-

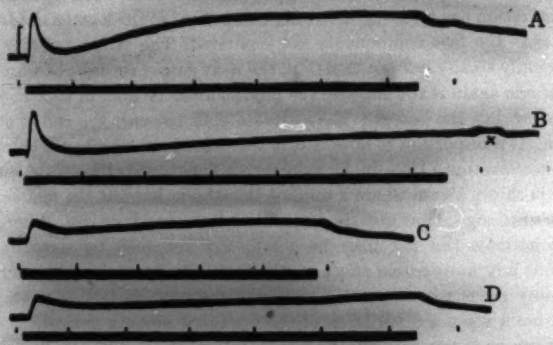

Fig. 3. Full description in text. Cross marks artefact following slightly increased off-effect.

zation will henceforth be termed the first process, P I. At times most of it disappears in 5 min. when there is still reflex activity left in the animal. Sometimes P I is more resistant.

Final effects of narcotization.

Further narcotization begins to affect the *b*-wave, which diminishes in height. When this happens the latent period, so far constant for a given intensity, begins to lengthen, and generally the rate of rise of the *b*-wave also becomes slower. This effect can be noted at all intensities. If the original intensity has been high the positive slow remainder after removal of P I often becomes replaced by a slow negative deflection. The final result is always that the whole response becomes negative, provided the intensity has been high enough to elicit a negative effect. A large number of such negative responses have been published by previous

workers [see below and Kohlrausch, 1931]. The early investigators, whose technique involved elaborate dissection, saw practically nothing but the apparently very resistent negative responses with mammalian eyes [see *e.g.* Holmgren, 1880; Dewar and McKendrick, 1873]. To the work of Kühne and Steiner [1881] we owe the knowledge that the response to white light should be chiefly positive [cf. also Dewar, 1876].

The negative response is influenced by ether just as is the *b*-wave. During continued narcotization its latency begins to lengthen, the potential diminishes and the rate of fall becomes slower. These effects can often be noticed before the *b*-wave has disappeared. The most

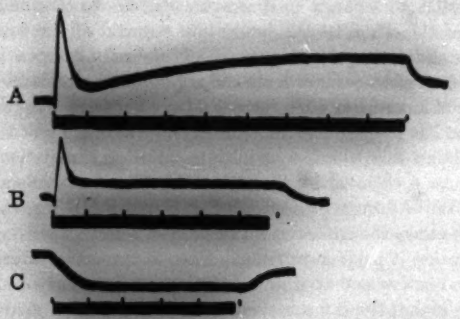

Fig. 5. Progressive effect of anæsthesia recorded at I/10. *A* is control before narcotization. *B*, after 21 min. of anæsthesia. *C*, after 31 min. of ether. Slack string, *b*-wave of *A* giving 0·417 mv.

marked alteration, however, found already at an early stage of anæsthesia, is that the return to the base line after cessation of the stimulus becomes sluggish. Fig. 4 (Plate I) shows part of a typical negative response of this type. All this makes it extremely difficult to intercept with the camera a negative wave at a moment when it can be proved to be uninfluenced by ether. An early negative wave was photographed in the experiment shown in Fig. 5. The intensity is I/10, the uppermost curve showing the normal response. The next curve shows a stage of anæsthesia where the positive remainder after removal of P I is already affected. The final negative wave in this case falls rapidly and also rises fairly rapidly at the cessation of the stimulus. It is larger than most of the negative waves seen in these experiments.

If the narcotic be removed at this stage of final negativity, and, if necessary, the animal be given some artificial respiration, complete recovery is generally possible. All the effects are thus reversible. Often the secondary rise is enhanced some time after recovery has taken place. Likewise when ether is first given there may be a stage of very short duration when P I is temporarily increased. Similar effects are also at times observed with the *b*-wave.

The final stage in progressive narcotization is complete disappearance of the response. This stage may mark an irreversible change. It has not been possible to revive the retina once it has ceased to respond.

By comparing ether effects at different intensities of stimulation it is again possible to separate into components the curve remaining after removal of P I. Thus, at low intensities, removal of the total positive remainder shows that this was practically the only component present, since no effect whatsoever or a minute negative response is all that is left at a stage of anæsthesia when there is a large negative deflection at high intensities. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities of stimulation and will, therefore, be designated by the term P II. The negative wave will be termed P III. All three processes, P I, P II and P III, react in a similar manner to ether, though a certain amount of selective resistance to the narcotic makes it possible to remove them in three characteristic steps. The normal action potential to weak stimuli consists of an almost pure P II. This makes the cat a very suitable animal for an analysis of the kind attempted in this paper, for any effect on P II can always be checked by using a low-intensity stimulus.

Removal of P II.

The fact that it is difficult to intercept a negative wave at a stage of anæsthesia when its latency can be proved to be unaltered may depend upon deficient selectivity of the narcotic with regard to P II and P III. It is, therefore, necessary to try some other means of removing P II from a high-intensity curve in order to determine the shape and latency of the negative wave, particularly with reference to the initial brief negative deflection. It is also necessary to test the analysis obtained from the ether results by some other method.

A case in point is illustrated by Fig. 6 (Plate II). *A* is a normal response to I. Then the carotid is occluded for 2 min. and *B* is obtained. The *a*-wave is slightly accentuated, the *b*-wave has become very small. It is now both followed and preceded by a negative deflection. P I is enhanced by the

asphyxia. The off-effect is definitely larger having been only a retardation in the post-stimulatory fall in the previous normal response. The animal has moved just before the light goes off. Troublesome movements generally occur when the carotid is occluded. The off-effect represents, however, a real increase, as has been confirmed in several similar cases, and may be seen in curve *H* in the same figure. *C* shows the initial part of the response to the same stimulus 2 min. later. Then the carotid is released, and 14 min. later a new record *D* is taken showing full recovery and persistence of the enhanced P I. The carotid is finally occluded once more, and a record *E* is taken 5 min. afterwards. Apparently then some collateral compensation has taken place, since the record is normal in form but shows diminished deflections. *F* shows a normal response to the same intensity in another experiment. Then the animal was curarized and *G* was taken showing one further stage in the process begun above by arterial occlusion. Curare may affect the *b*-wave to some extent—I have seen it do so—but the curve may still be of the normal type [Kohlrausch, 1918]; there was, however, a serious leakage in the tube leading to the pump of the respiration apparatus, and this must be the explanation of the almost complete removal of the *b*-wave. The *b*-wave is only a retardation in the negative deflection initiated by the *a*-wave, and the initial negativity *a* runs almost directly on into the large negative P III. The latency is constant, about 20σ in both records.

Curare was used to induce asphyxia without troublesome movements in the animal. But it was deemed unnecessary to continue these experiments since the question appears to have been settled by Kohlrausch [1918], who used a curarized rabbit (rod-eye). The results obtained by arterial occlusion may just as well be brought about by stopping the respiration in a curarized rabbit. Kohlrausch accidentally noted a response of the type shown in *B*, *G* and *H*, and then found that the respiration apparatus did not function properly. Adjustment of the artificial breathing brought the response back to normal again. His deficient curve shows no *b*-wave, an enhanced secondary rise and an increased off-effect. It looks, in fact, precisely like curve *H* in Fig. 6 (Plate II)[1].

The deficient response *H* was the result of an unsuccessful operation. Probably the carotid was occluded too long, since this in some animals leads to irreversible disappearance of *b*. In this case the animal gave the same type of response for some time. In other similar cases there either appeared later a small *b*-wave or the response diminished in amplitude

[1] In later experiments, some of which are mentioned in Part II, asphyxia after occlusion of the carotid sometimes completely removed P II.

fairly rapidly. When in such cases ether was given, removal of the secondary rise left a pure negative remainder. In order to find out by some other means whether the second phase of P II, which in high-intensity curves is covered by P I, is lacking in responses of this type, the 100 times lower intensity was used. Curve *I* gives the response to this stimulus, adjusted to the same light signal as *H*. Of the considerable negative practically nothing is left at this intensity, and of the large secondary rise there is but a fraction. This wave has a very long latency just as P I. The fraction left also corresponds to the amount of P I that the ether analysis (cf. Fig. 3) showed may be present at this intensity. P II at this intensity is much larger and quite different as clearly shown by Fig. 1, curve *C*. The negative P III also behaves with respect to intensity as was to be predicted by the results obtained with ether. Evidently then P II in addition to the *b*-wave contains a second positive phase, which is practically pure at low intensities. At these intensities P I and P III are small or absent. The response *H* is a combination of P I and P III.

Components in relation to stimulus.

By using ether it was possible to obtain P II alone at low intensities, P III alone at high intensities, and also to produce the response P II + P III. By interfering with the oxygen supply it was possible to produce the response P I + P III. It has not been possible to obtain P I alone. Thus it follows that P II and P III are very directly related to the stimulus. It does not seem probable that the one elicits the other. As to P I, it may, of course, merely be a matter of finding the right procedure in order to obtain it alone, but so far it seems as if P I required P III. It does not require P II to judge from the large P I in the response P I + P III.

P III in relation to a- *and* d-*waves.*

The evidence so far obtained allows certain conclusions to be drawn regarding the place of the *a*-wave and the off-effect in the analysis of the composite effect. The ether experiments (cf. Figs. 2, 3) often show an enhanced off-effect after removal of P I. Thus P I is not necessary for the off-effect to appear. On the other hand, removal of P II at high intensities regularly increases the off-effect as confirmed by Kohlrausch's similar findings. Therefore P II cannot cause the *d*-wave. This also follows from the low-intensity curves which contain a large P II and practically nothing else. They never show an off-effect. Thus the component necessary for the *d*-wave to appear is the negative P III and, in addition, either P I or P II to serve as a background against which the return to zero of P III

at the end of stimulation can set itself off. For the alternative explanation that P III actually rises above the base line at the end of stimulation there is no evidence. The purer the negative waves the more definitely are they monophasic. Since P I and P II drop at the cessation of the stimulus, it follows that the absence of either counteracting fall must make the off-effect caused by the return to zero of P III more marked. P II drops faster than P I. Therefore, the removal of P II must cause particularly large off-effects which actually is the case. Kohlrausch [1918], using Piper's analysis, gives the same explanation of the large off-effects after removal of P II by interfering with the artificial respiration. The experiments reported above settle the fact that the off-effect depends upon the behaviour of P III.

The negative waves obtained after ether in general possessed too long latencies to have started before the positive P II, though, on the other hand, the effect of the narcotic upon the latency made it probable that, if a negative could have been obtained at a sufficiently early stage of anæsthesia, it might well have started before the positive wave. The experiments on removal of P II by interference with the blood supply gave the additional information wanted. A series of records was obtained showing the initial *a*-wave gradually running on into the large negative P III. This time the latency was not altered, but remained at about 20σ, the value of the latent period of the *a*-wave. Not only, therefore, is it unnecessary, but it is also unreasonable to assume a separate twitch-like initial *a*-wave when all the evidence shows it to be closely related to the large negative P III. Thus it is influenced by area and intensity just as P III, and is lacking in the pure P II responses.

Previous analyses will be mentioned below, but in this connection some of the facts relating to negative waves deserve to be pointed out separately. Thus Piper [1911], confirmed by Kohlrausch [1918], found that vertebrates (fishes, amphibians, reptiles) which had large *a*-waves also had good off-effects, whereas those (certain mammals as rabbits, cats, dogs) which gave small and inconstant *a*-waves also had small or inconstant off-effects. These two, the *a*- and the *d*-wave, must therefore be ascribed to the same process, which above was shown to be P III. The same conclusion was reached by Einthoven and Jolly [1908]. Waller [1909], confirmed by Jolly [1909], obtained fast monophasic negative waves by giving frog's eyes massage. Waller found these negatives to possess a shorter latency than the positive deflections. The return to zero was also fast. These facts he incorporated in his analysis [cf. also Einthoven and Jolly, Piper, Kohlrausch].

Nikiforowsky [1912] obtained negative waves by cooling frog's eyes. He, as later Tirala [1917], found that the *d*-wave cannot be due to the same process that causes the *b*-wave, above demonstrated to be P II.

The analysis of the composite effect.

The analysis of the retinal action potential in the dark-adapted cat's eye may now be given with a fair degree of confidence. There are three processes numbered in the order of their disappearance during progressive anæsthesia. Their relative contributions to the composite effect varies with area and intensity as set forth above. Since an increase in area affects the response just as an increase in intensity, it is only necessary to solve for two intensities. Besides, the effect of area, owing to effects of

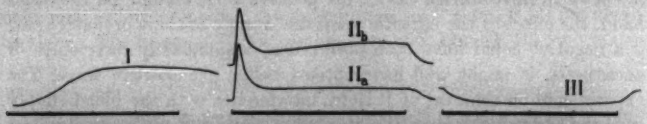

Fig. 7. Components of retinal action potential to full I for stimulus lasting 2 sec. Two alternatives are given for P II. For P II, as in II *a*, P III should be constructed rising as II *b* to fit cases in which, after removal of P I by ether, the composite potential still shows a secondary rise.

shunting by the tissues, cannot be as reliable an index of what takes place as the effect of intensity. The low-intensity response is simpler and the components are therefore only given together with the composite curve in Fig. 8. The high-intensity components are also given separately in Fig. 7.

Since the high-intensity second process, P II, cannot be obtained separately at this intensity, it is evident that the rise which sometimes is observed after removal of P I may be solved from a rise in P III or a rise in P II itself. Isolated third processes have sometimes in the course of this work been found to rise slightly during continued illumination. If this rise in some animals were large enough it could account for the rise observed after removal of P I. That would necessitate a P II of the type *a* in Fig. 7. The same type of P II would be obtained if for some reason in certain animals P I were unusually resistent, though the evidence with low-intensity second processes does not support this view. The same experimental response, P II + P III, after removal of P I could also be obtained if P II itself rose slightly as shown by curve *b* in Fig. 7. The latter alternative finds some support in evidence to be presented in Part II.

There the components will be evaluated in terms of processes in the optic nerve. The composite curve of Fig. 8 is constructed on the basis of a P II of the type *b*. The figure summarizes the results of the analysis carried out above and needs no further comment. It may, of course, be constructed to give a larger off-effect after removal of P I.

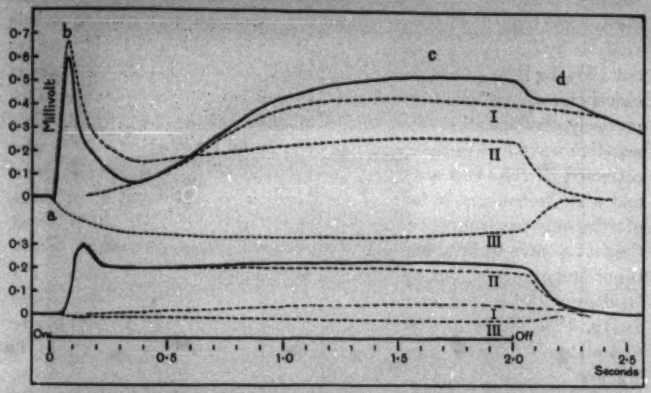

Fig. 8. Analysis of composite retinal action potential at two intensities, 14 ml, and 0·14 ml, and area of 1661 sq. mm. viewed at a distance of 70 mm. Components: broken lines. Composite curve drawn in full. The *a*-wave is broadened slightly out of scale to show its derivation more clearly.

Previous analyses.

In view of the fact that the retinal action potential for various vertebrate eyes contains the same phases and that histological evidence shows all such retinæ to be built on a similar plan, the analysis must be general in principle. It therefore becomes particularly interesting to compare it with the two most important analyses, *i.e.* those by Einthoven and Jolly [1908] and by Piper [1911]. Behind these two attempts there is not only a many-sided experimental experience [Piper], but also systematic analysing of response curves obtained under various experimental conditions [Einthoven and Jolly], [Kohlrausch, 1918]. The slow instruments used by the early workers in this field, often in connection with severe operations, made delicate work impossible. A notable exception is the very interesting contribution by Kühne and Steiner [1881].

Einthoven and Jolly recognized the three processes, or "substances" as they call them. P I is quite correct, P II lacks the slow second phase showing that they did not realize that the *c*-wave is not homogeneous. P III gives the negative as a twitch below the base line and the off-effect as a positive deflection. The intervening negative phase is lacking. It is important to note that they found that the *a*-wave and the *d*-wave had to be due to the same component. Piper improved upon this analysis by introducing the intervening negative phase of P III, and by not allowing it to end in a positive process. In fact, his negative wave is strictly monophasic and the off-effect as well as the *a*-wave are solved by precisely the same interference construction as above in Fig. 8. This negative wave had in the meantime been found by Waller [1909], who corrected Einthoven and Jolly's solution in this respect. He resolved *d* and what he believed to be *a*, as did Piper later, but made the mistake of using only two components, a positive and a negative. He also believed the fast *a*-wave to be abnormal. Piper further realized that the *c*-wave is not homogeneous, but contains P I and the second phase of P II. P I is identical with the corresponding "substance" of Einthoven and Jolly. P II differs from the analysis given above in that it rises rapidly to a maximum which then is retained during continued stimulation. It is a low-intensity P II added to high-intensity first and third processes. This P II was based on his experiences with the Cephalopod eye [1911] which, of course, is analogous only, not homologous with the vertebrate retina. It is possible that in some animals the fall of the *b*-wave is mainly due to P III rather than to a decline in P II. In the cat at high intensities this certainly is not the case. Evidence to be presented in Part II makes it somewhat doubtful whether it can ever occur at such intensities. Still, Piper's analysis is essentially correct, though largely hypothetical, and the general interest taken in Einthoven and Jolly's and Piper's combined efforts to solve the retinal action potential has not only been fully justified by the work presented in this paper, but also by Kohlrausch's experiments with various colours. He there found that the short wave-lengths produced very small second processes followed and preceded by negative deflections, resembling, in fact, curve *B* of Fig. 6. The long wave-lengths, on the other hand, had a greater effect upon P II. Both lights together gave summed curves, which not only corresponded well with the theoretical composite curves but could also be explained on Piper's solution. Most important was the finding that partly negative deflections can be obtained in, for instance, a cone-eye like the pigeon's by stimulating with short wave-lengths. This rules out the supposition that a

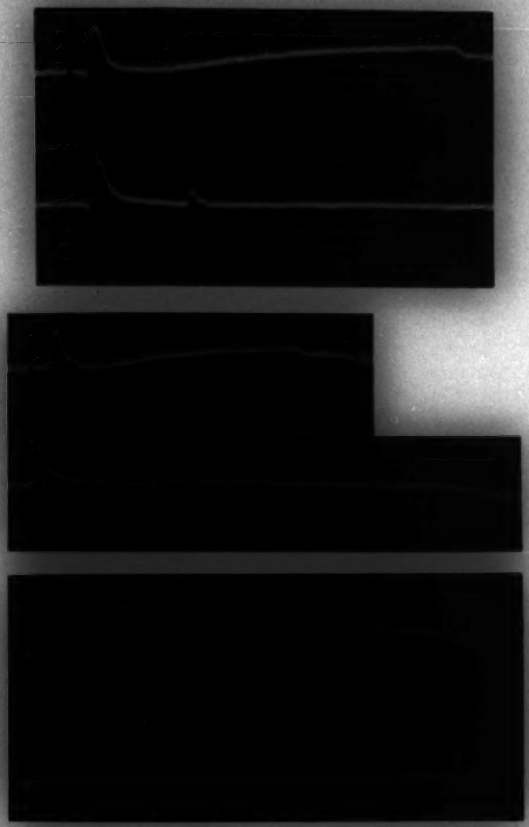

Fig. 2. Three deflections to full I during initial stage of light anæsthesia together with controls taken before narcotization. Full description in text. Artefact marked by cross is probably movement in animal's eye.

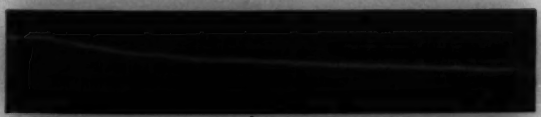

Fig. 4. Negative deflection to full I obtained after prolonged narcotization.

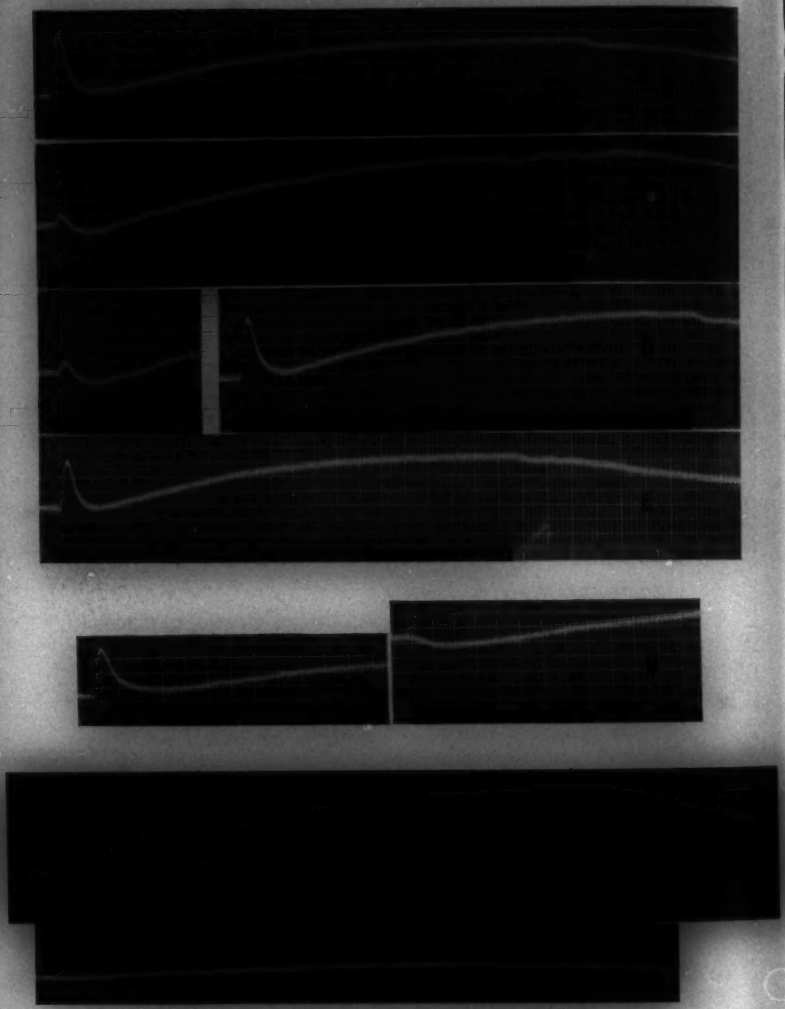

Fig. 6. Full description in text. Deflection in bottom record, to save space, adjusted to light signal of foregoing record. Arrow marks end of stimulation.

Fig. 9. String galvanometer records of retinal response and oscillograph records taken from the optic nerve. Time: tuning-fork, 100 per sec. Signal is seen causing escape at on and off of light, picked up by oscillograph. Explanation in text.

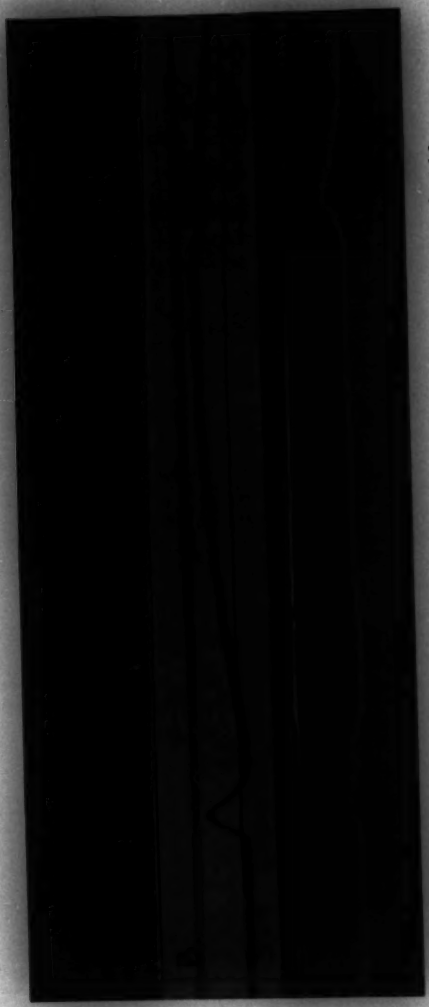

Fig. 10. *A* and *B*: string galvanometer records of retinal action potential. In *A* stimulus lasts about 1·3 sec., in *B* about 2·5 sec. *C*: oscillograph record from optic nerve, stimulus lasting about 1·3 sec.

retina with negative deflections is pathologically changed. The perfect recovery from the negativity obtained above is also difficult to interpret on such a basis.

The discussion of the nature and localization of the components will be postponed till evidence relating to the optic nerve has been presented.

Summary.

Leads from the cornea and decerebration wound have been taken to the input of a directly coupled amplifier with a string galvanometer in the output. The aim of the work has been to try to establish a biological analysis of the complex action potential of the retina. This has been done in two ways: by giving the animal ether and by interfering with the blood supply of the retina. Both agents were found to affect certain components selectively and in a reversible manner.

Narcotization removes in three characteristic steps definite components of the response to stimulation with white light. These components are indicated in Fig. 8 by Roman letters in the order of their disappearance and given separately for a high intensity in Fig. 7. Process I (P I) disappears rapidly during narcotization and the fast deflections are left unchanged. It is essentially a high-intensity component. Thus, at an early stage of anæsthesia, this component may be minute or even absent at high intensities, whereas the low-intensity response is almost or even completely unchanged. Therefore the slow phase of the composite effect is not homogeneous. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities, diminishing gradually during continued anæsthesia. This component is termed P II. Finally only a negative, P III, is left provided the intensity has been high enough. The last stage is a gradual disappearance of P III. The ether analysis shows the response at low intensities to be a practically pure P II. Removal of P I need not affect it, and when the positive deflection is removed there is no negative left.

Asphyxia in the animal or occlusion of the carotid affects selectively P II. The selectivity may be demonstrated by testing with the practically pure P II at a low intensity. The high-intensity response contains P I and P III, and is a large negative deflection followed by a secondary positive rise.

Removal of P II in this manner shows the brief initial negative (*a*-wave) running on into the large negative P III of which it is therefore a part.

Removal of P I by ether often enhances the off-effect. Removal of P II by asphyxia regularly enhances the off-effect. The practically pure P II at low intensities never gives an off-effect. Therefore the off-effect depends primarily upon P III. Since, however, P III produces an off-effect only in the presence of either P I or P II it must be resolved by an interference construction from the rise of P III (cf. Fig. 8).

Part II. The latent period and the relation between the processes in retina and nerve.

Action currents from the optic nerve were first successfully recorded by Kühne and Steiner [1881], later by Ishihara [1906] and by Westerlund [1912]. The effect obtained resembles the retinal action potential, even the initial fast *a*-wave being present in the records of Westerlund. In none of the records published can a secondary rise (*c*-wave) be found. Frōhlich [1914] observed upon the retinal action current of the cephalopod eye oscillations which have been interpreted as caused by impulses in the optic nerve, but there are also other explanations to be considered [cf. Kohlrausch, 1931].

The actual impulses in the optic nerve were then recorded in an interesting work by Adrian and Matthews [1927 *a*, *b*, 1928], who used a capillary electrometer and an amplifier. They used the long optic nerve of the conger eel. Adrian and Matthews confirmed the general relation between intensity of stimulation and frequency of discharge, established by Adrian and his successive collaborators [cf. Adrian, 1928] for various sensory end organs and neurones. They also obtained the frequency-time curve of the retinal discharge. We now know that the frequency of the impulses discharged by the retina first rises rapidly at the onset of stimulation, then falls to a lower level during continued stimulation, and also that the off-effect of the retinal action potential has its counterpart in a renewed outburst of impulses at the cessation of illumination. Considering the slowness of the instruments used by the early workers it is possible that what they recorded was the integrated total frequency-time curve, obtained by Adrian and Matthews by plotting the impulses per unit time against time of stimulation. But it is also quite probable that the effect recorded was due to spread from the retinal currents. The latter view appears to be taken by Westerlund, and my own experiences with "integrative" recording controlled by

oscillograph records taken with large condensers in the amplifying circuit show that "integrative" records may be seriously distorted by retinal effects, at least when the leads are applied as will be described below.

Most important is the observation by Adrian and Matthews that the off-effect also is translated into impulses. This distinguishes the retinal discharge from that of other sensory end organs recorded by Adrian and his co-workers [Adrian, 1928]. Interesting work with the *Limulus* eye has recently been published by Hartline and Graham [1932], who succeeded in obtaining impulses from a single ommatidium. The ommatidium is a fairly complicated structure [Demoll, 1910; Versluys and Demoll, 1922–3], but is not connected with other ommatidia by way of internuncial neurones. However, its internal organization is complicated enough to make it appear questionable whether it can be assumed to be non-synaptic. The retinal action potential of several ommatidia looks like the isolated component P II of the cat's eye and appears to be related to the frequency of the discharge in the nerve [Hartline, 1932]. Further experimentation, no doubt, will show whether it is homogeneous or contains a hidden component of opposite sign and whether this eye gives an off-effect.

In this work the aim is to gather information as to how the components of the retinal action potential, isolated in Part I, are represented in the optic nerve. It has not been possible to accomplish this in a quantitative manner. The cat's optic nerve is rather unaccessible and easily damaged. In order to ensure satisfactory development of all three components of the action potential a great number of fibres must be activated which further complicates the task of recording. But the choice of preparation is fully justified by the fact that the retinal action potential of the decerebrate cat is easily split into components.

Method.

For retinal responses the technique has already been described in Part I. The "push pull" battery-coupled amplifier was used in most cases; in later work a new two-stage amplifier, also battery coupled, built on the principles set forth by Chaffee, Bovie and Hampson [1923], was used. With Mazda Pentodes 220, this system gives a base line free from drift and a total amplification of about 50. This is more than needed for work with eyes of decerebrate animals. The same amplifier and string galvanometer were used for obtaining records from the optic nerve with syringe needle electrodes [Adrian and Bronk, 1929], stuck into foramen opticum from the cranial side [Granit, 1932 *a*].

When impulses were recorded the animal in its well-insulated and shielded box was moved into another research room where a Matthews' oscillograph with its amplifying system was set up for other purposes. A Cambridge string galvanometer could be worked alongside the oscillograph, and sometimes this string was also connected to the directly coupled amplifier described above. The stimulating and signalling system could not be shifted as easily as the preparation, and therefore a small lamp, run from an 8-volt accumulator and adjusted by means of lenses to illuminate a large part of the retina, was used in connection with the oscillograph. Records of the retinal action potential showed this illumination to be of the order of magnitude of the high intensities obtained with the other apparatus (cf. Part I). The electrodes were generally silver pins. The two leads were used in various positions relative to one another, but the best results were generally obtained when they were parallel and stuck in obliquely deep into the foramen opticum. The discharge recorded in this manner consists of regular or irregular oscillations dependent upon the degree of synchronization in the fibres concerned. Naturally this index of nervous activity is qualitative rather than quantitative, but some idea about the intensity of the effect can be gained by considering various aspects of the records. A test on artefacts was provided by the fact that the experiments ended with removal, sometimes accompanied by restoration, of the components of the retinal action potential.

The stimulating light was generally switched on by means of a key in its own circuit. This moment was recorded on the plate by a pointer attached to a magnetic short-circuiting device. But in some cases a photographic shutter was employed, and then the on and off of the stimulus were not recorded. In the former case the heating and cooling time of the filament entered into the latency of the on- and off-effects. This, of course, was not the case when the accurate device used with the apparatus described in Part I was used. However, when oscillograph and string galvanometer were worked together an absolute value for the latent periods was not needed, the purpose of this combination being to compare retinal and nerve responses relative to one another. Altogether some fifteen animals were used.

Retinal processes in relation to nerve discharge.

Adrian and Matthews [1927 *a*] also made a first attempt to study the mutual relation between retinal and nerve response. They were, however, seriously hampered by the fact that their condenser-coupled amplifier could only reproduce fast processes correctly; hence, as they

point out, the comparison between retinal and nerve response had to be restricted to the initial and final phases of the two processes. They found that the impulses started during the *a*-wave and that the retinal nerve interval was roughly constant. The latter interval was measured from the beginning of the *a*-wave. They concluded that the initial negative deflection is closely associated with the initial outburst of impulses. That the off-effect in the retinal action potential was found to be correlated with an outburst of impulses has already been mentioned.

In view of the fact that the retinal action potential, as shown in Part I of this paper, is initiated by two processes of opposite sign, their evidence with regard to the *a*-wave hardly allows any conclusions as to which component is associated with the discharge in the nerve. Either the positive component P II, responsible for the *b*-wave, or the negative P III, responsible for the *a*-wave, or both, may be concerned with the initial outburst of impulses. P II may be present at the onset of P III, or P II, if alone assumed to set up impulses, might also start at a constant interval from the beginning of the *a*-wave. It is evident that no records of the complex retinal action potential give any information on this point. The latent period of the *b*-wave merely shows where the rise in P II has cancelled the fall in P III. This moment is probably of some significance in an interpretation of the retinal action potential, but it cannot throw any light upon the relation between the retinal processes and the discharge in the nerve. There was therefore no object in trying to determine the latency of the initial outburst of impulses relative to the beginning of the *a*- and *b*-waves, the less so as in the cat the *a*-wave is small and present only at high intensities. In so far as the onset of the discharge could be measured from records involving a certain amount of synchronization of impulses, it was found to be almost coincident with the onset of the *b*-wave, or to follow not later than 10σ after the first manifestation of a positive deflection, provided that the retinal potential was measured with the string connected to the amplifier.

Not only does indirect evidence support the view that the discharge in the nerve is associated with a positive component, but it also renders it difficult to assign a similar function to the negative wave. One difficulty deserves to be pointed out. The analysis of the retinal action potential (cf. Part I) indicates that the positive off-effect is a release of the positive components from the negative wave, following the return of the latter to zero. Now the retinal off-effect is definitely connected with a discharge of impulses through the nerve [Adrian and Matthews]. If now the *a*-wave, representing the beginning of the negative wave, is

assumed to set up a discharge, it does not seem possible to account for the fact that thereby the negative component is assumed to increase the frequency of the discharge both when P III increases (*a*-wave) and decreases (off-effect).

The only way in which at present these questions can be approached appears to lie in careful consideration of the various phases of the retinal action potential and how they may be brought to conform with the picture obtained from the nerve. The latter should at least give information on two points: (i) Is there a secondary increase in the discharge corresponding to the large secondary increase in the retinal action potential due to P I (*c*-wave)? (ii) How does removal of the positive components of the retinal action potential affect the discharge through the nerve?

Fig 9 (Plate III) shows what happens when a purely negative response is produced by interfering with the other waves by the methods described in Part I. Unfortunately, in this case a very small retinal response was obtained with a slack string without an amplifier, but the experiment has the advantage of illustrating both the effect of ether and asphyxia, and also recovery from negativity, in the same preparation. The condensers in the amplifying circuit of the oscillograph were 0·02 microfarad. *A* shows the retinal response at the full intensity; the secondary rise is small. *C* shows the corresponding oscillatory discharge in the nerve. The tendency of these oscillations is to diminish in amplitude during continued stimulation; the frequency may diminish but need not necessarily do so. Values between 100 and 150 oscillations per second are commonly found, the lowest regular frequency noted has been 80. Evidently the discharge consists of synchronized impulses [cf. Adrian and Matthews, 1928; Adrian, 1932]. The frequencies are higher than those noted by Adrian and Matthews in the eel's eye with large areas. If the oscillations noted by Fröhlich are to be similarly interpreted, it is to be observed that the effect of a diminution in intensity primarily affects the amplitude of the oscillations, whereas the frequency decreases but little if at all. Thus *B* is the retinal response at a 100 times lower intensity and *D* the corresponding nerve response, illustrating the diminution in amplitude. Fröhlich found the frequency of the oscillations to increase and decrease with intensity of stimulation. The off-effect in the nerve records is small in this particular experiment, but again the increase in amplitude of the oscillations is noticeable. Thus with large areas in the dark-adapted cat's eye the amplitude of the oscillations is the most definite index of a change in intensity of the retinal effect,

though by no means as good as direct counting of the frequency of the individual impulses where this can be done. It should be noted that there are no indications of a slow secondary rise in the amplitude at the high intensity though the retinal response *A* definitely rises during continued stimulation.

Then the animal is given ether heavily, and in 5 min. the retina has lost its positive components. *E* shows the slow negative deflection in the string. *F* is the nerve record taken immediately afterwards. No oscillations are visible. The anæsthesia is interrupted and 8 min. later the retina gives the response *G*. The oscillations have returned as shown by *H*. Then the carotid is occluded and the negative response *I* is produced in less than 1 min. This is larger than the positive response at the low intensity (*B*). Yet, again no oscillations are visible, though the nerve record, *J*, is taken but a few seconds after the retinal response on the same plate.

Both methods of removing the positive components thus lead to the same result: the impulses disappear. Agents that block the passage of impulses also block the positive components of the retinal action potential. These two processes in retina and nerve respectively are thus closely interrelated. Whether this means that the impulses actually are set up by a positive component or only that the positive components and the oscillations in the nerve are two aspects of the excitatory process as expressed by physiologically and histologically different structures, is at present a secondary question, to be solved, if possible, with different methods.

It is further evident that of the two positive components P II undoubtedly is concerned with the discharge through the nerve. The impulses appear long before there is any sign of the slow secondary rise (*c*-wave) of P I, and, even though the latter is small in this experiment at the high intensity, there is nothing at all to indicate an equivalent rise in the amplitude of the discharge. The off-effect in the cat's retinal action potential is generally only a retardation in the drop of potential following cessation of stimulation, yet this is nearly always accompanied by a corresponding increase in the amplitude of the oscillations. In Part I it was pointed out that the off-effect on the response P II + P III, from which P I had been removed, at times was found larger than in the complex response owing to the fact that in the latter it was compensated for by the fall in P I at cessation of stimulation.

The isolated negative response cannot be shown to set up impulses. This may be accounted for by a block caused by ether or asphyxia, an

explanation difficult to exclude, but not altogether satisfactory. Observations mentioned in Part I showed that an alteration in the amount of potential developed by any component was preceded by a change in latent period. It was further found that a short asphyxia, as in this experiment, did not change the latent period of the negative component. Thus, by this method P III is obtained in as nearly normal form as possible, as indicated by the relatively large negative deflection *I* (Fig. 9). Considering that the impulses disappear and reappear with P II it is difficult to assume that a negative wave, which hardly is influenced at all by the same amount of asphyxia (1 min. occlusion), should be responsible for the discharge in the optic nerve.

Fortunately the analysis of the retinal action potential demonstrates the nature of the process behind the negative wave, provided that we know the function of P II. This, as we have seen, is to set up impulses or to be a link in the chain of events leading up to the discharge through the nerve. The analysis gives the further information that the off-effect is a release phenomenon, caused by the return to zero of the negative wave. The evidence may be found in Part I. This would be merely an algebraical fact—and is probably so with respect to the response P I + P III (see below)—if we did not know that P II is concerned with the discharge and that the off-effect also is accompanied by impulses. Knowledge of all these facts leads to the conclusion that, when P II is released from the negative P III, it is actually being released from something which not only did not produce impulses, but actively inhibited them. P III can act in this manner only if in some way it is connected with an inhibitory process. The off-effect must then be a true "post-inhibitory rebound," the term signifying a discharge following as a release from an inhibition. Further evidence bearing on these conclusions will be presented in a subsequent paper by Dr R. S. Creed and the author.

In order to stress the significance of possessing the correct analysis of the retinal action potential and what is chiefly an application of this knowledge to the present problem, the following facts might be pointed out: the *b*-wave and the off-effect are both positive in the retinal action potential, and both are concerned with the discharge through the nerve. In addition the off-effect has also been shown to depend upon the negative wave (cf. Part I). All these facts are taken account of by the above deductions which also are in accordance with the fact that the retinal action potential arises in the synapses (see below).

As to the slow secondary rise given by P I it has already been

mentioned that previous work with the optic nerve has failed to give any indication of a corresponding increase in the frequency of the impulses in the optic nerve. Likewise in this work it has been noted that, whereas a small positive off-effect, visible only as a retardation in the drop of potential at cessation of stimulation, has been accompanied by a definite increase in amplitude of the oscillations, nothing of the kind has been observed with *c*-waves of considerable magnitude. An especially large secondary rise was found in the experiment illustrated in Fig. 10 (Plate IV). *A* and *B* show the retinal action potential at two speeds of the plate. *C* shows the response obtained from the nerve. This was taken with large condensers in the amplifying circuit, combined with a grid leak such as to cause a constant potential applied to the input of the amplifier to drop in the output to half its full value in half a second.

The question arises whether the initial deflection in the nerve and the large off-deflection are artefacts from the retina, or whether the discharge is partly monophasic, in which case the height of the deflection would roughly indicate the frequency of impulses out of phase. Both slow changes ultimately develop into the typical synchronized discharge. Independently of whether the amplitude of the synchronized oscillations alone or these together with the slow rise are interpreted as indicative of the intensity of the effect in the nerve, it is evident that the large secondary rise in the retina has no equivalent effect in the nerve. In this respect it clearly differs from the positive effects in the retina at on and off. Whichever index is used, the off-effect, though only a retardation in the drop of potential at cessation of stimulation, is followed by a marked increase in the intensity of the effect in the nerve. The small secondary rise, to be seen in this nerve record, could not be obtained in the other experiments of the same series. Yet, in one the illumination was allowed to act for over 2 sec. Similarly after removal of P I with ether no definite change can be found, though for such experiments the method is hardly sensitive enough. In some records there has been a small early secondary rise in amplitude which, however, has persisted after removal of P I [cf. Hartline and Graham, 1932]. This rise may be given by P II (cf. Fig. 8, Part I).

The latent period.

A theoretically very important experiment on the latent period was made by Adrian and Matthews [1928]. These authors confirmed an observation, previously made by Ishihara [1906], that an increase in area shortens the latent period just as does an increase in intensity. Actually the latent period of the optic nerve response was measured, but

since the retinal nerve interval was found roughly constant, the process responsible for the shortening of the latent period could be ascribed to the period preceding the retinal response. This latter latency was measured directly by Ishihara. But Adrian and Matthews also added an interesting analysis of this fact by proving that the spatial effect on the latent period could be obtained when areas some distance apart on the retina were stimulated. The latency of the nerve discharge for four lights acting simultaneously was found to be shorter than that for each spot alone. In addition they proved that stimuli so far apart as not to interact could be made to do so by application of strychnine to the retina. This not only excludes any explanation based on scatter of light but also shows that the lateral connections between cells in the retina react to strychnine just as similar connections in the central nervous system. In an eye lacking the internuncial neurones of the vertebrate retina the spatial effect should be absent. Adrian and Matthews' analysis of the spatial effect may therefore be held to have been significantly verified by the observation by Graham—kindly communicated to the author from work in course of publication—that in the eye of *Limulus*, which lacks internuncial neurones, the influence of area on the latent period is absent, though the intensity effect is present.

The spatial effect is of such fundamental importance because it localizes the process responsible for the action potential to a point in the retina which is synaptic or post-synaptic. The first lateral connections are lying around the synapses between receptors and bipolar cells. Thus, since the synaptic effect is to precede the response, the action potential cannot be localized to the rods and cones themselves, but must be produced at the point where the receptors are joined to the bipolar cells or later. There cannot therefore be more than one synapse between the retinal action potential and the impulses in the optic nerve.

This experiment by Adrian and Matthews has been confirmed with the flicker method and the human eye [Granit, 1930], but it was thought desirable to repeat it with the action potential of the cat's eye as well. Four circular discs, 6 mm. in diameter (about 56′ of visual angle), were placed symmetrically as outer tangents to an imaginary circle 19 mm. in diameter (nearly 3°) at a distance of 370 mm. from the cat's eye. The intensity was 11·2 millilamberts. The average latency of response to the individual lights in nine determinations was found to be $67 \pm 2{\cdot}3\sigma$ mean variation. All four together gave $59 \pm 2{\cdot}0\sigma$ as an average of five determinations. The decrease in latency is thus 11·9 p.c. of the value obtained with the "singles." The latencies were measured with a magnifying glass

placed over a scale reading 0·1 mm., the speed of the film averaging 8–9 scale divisions for 10σ.

In order both to compare this effect with the corresponding variation caused by an increase in area and to find out how much could conceivably be accounted for by scatter of light, the area was varied in the same experiment between limits of 4·5 and 46 mm. in diameter, other conditions being equal. Three readings were taken for each area. The latent periods are plotted in Fig. 11 against log area. The relation is well

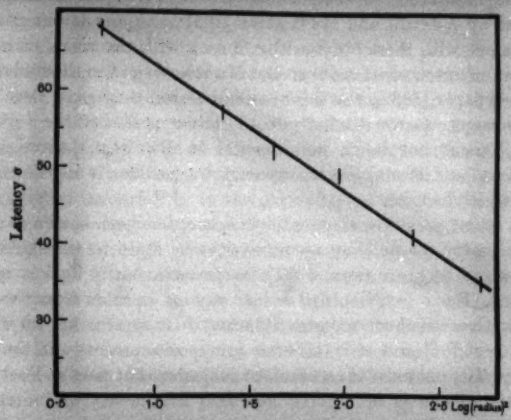

Fig. 11. Ordinates: latent period of *b*-wave of retinal response (no *a*-wave visible). Abscissæ: log area of stimulus. The readings are marked by lines corresponding to ordinates of 2σ. Explanation in text.

represented by a straight line, the one drawn being calculated from the data by the method of least squares. From this graph or from the equation could be read the decrease in latency corresponding to a quadrupling of the area stimulated, given by the four spots together over the area of each single stimulus. The effect evaluated as above is 15·4 p.c. as compared with 11·9 p.c. obtained with separated stimuli. Thus about 77 p.c. would have to be accounted for by scattering of light. This appears an improbable figure, even if the strychnine experiments were not there to disprove this hypothesis.

Intensity influences the latent period in a similar manner [Einthoven and Jolly, 1908; Ishihara, 1906; Adrian and Matthews, 1927 *a*;

Granit, 1932 *b*]. But within the range of intensities used in these experiments it has always been possible, by diminishing the area, to reach a value of the latency, which could not be compensated for by an increase in intensity. This means that the individual receptor-bipolar synapse at any intensity must have a longer latency than any recorded. The value obtained is largely dependent upon the amount of spatial summation which occurs, that is, upon the area stimulated. The negative initial wave, as pointed out above, also complicates the picture. It is therefore misleading to compare, as Hecht [1931] does, the results obtained by Adrian and Matthews [1927 *b*] on the latent period of the eel's eye with those obtained by himself with the visual end organs of certain invertebrates. Adrian and Matthews did so themselves, but in a later paper [1928], the third in their series, they gave clear proofs that a synaptic factor entered into the latent period of the vertebrate retina. This is not taken into account in Hecht's theoretical considerations, and it may be unnecessary to consider it in the receptor organs which he used.

The latent period in a synaptic structure has been shown to be the time necessary for building up an excitatory state to threshold value [Eccles and Sherrington, 1931], in our case, partly against adverse inhibition. But it is difficult to deduce any information from the latent period of the retinal action potential when it is not even known whether the whole of P II and of P III refers to opposite processes in the same neurones. The presence of an off-effect indicates that part at least of the inhibitory effects is influencing neurones in the act of building up excitation.

Summarizing conclusions.

In the following summary the term "component" (sc. of the retinal action potential) is often substituted for "process," as has been done throughout this paper. But it is important to realize that when two components, measured by the amount of potential produced, are equal, this does not necessarily imply, for instance, that the processes eliciting those potentials are of equal strength. Not only does the picture obtained with the galvanometer depend upon the manner of leading off from the preparation and upon the sensitivity of the instrument employed, but it is also at present impossible to determine whether, to take an example, the frequency of the impulses is proportional to the deflection of the galvanometer with a pure P II or whether it is some more complicated function of the recorded potential. The same holds for P III. A more suitable preparation than the decerebrate cat would appear to be needed for a

study of these questions. It should further be recalled that the traditional way of representing the retinal action potential reverses the sign of the potential with respect to the retinal layers.

The complex retinal potential arising in the retina on stimulation with white light has been found to develop in that part of the sense organ which histologically is a "true nervous centre" [Cajal]. It appears after a latent period involving synaptic interaction, and hence cannot have arisen distal to the locus where the first synapses occur. The complex effect is an algebraical sum of three components, the properties of which may be summarized as follows:

The first process, P I (see Figs. 7 and 8, Part I, pp. 220 and 221), rises slowly after a long latency and falls in a similar manner. It is positive in the usual representation of the retinal action potential. P I is easily removed by ether. Before it begins to diminish it may, however, pass through a temporary stage of enhancement. Likewise after removal of the narcotic it is often temporarily enhanced in good preparations. Slight asphyxia often favours P I. In the dark-adapted cat the first process is only present with large areas and high intensities of stimulation. Observations by Kohlrausch [1918] on the *c*-wave of the complex potential indicate that P I in nocturnal animals is more marked in the dark-adapted eye, in diurnal animals in the light-adapted eye. In order to account for these facts Kohlrausch suggests that this wave appears whenever an eye functions under conditions most appropriate for the particular retina in question [Kohlrausch, 1931]. The dependence of P I upon a large area and a high intensity also suggests that this component appears when the retina is especially active. This component is not at all or only slightly concerned with the discharge of impulses. But it might well represent some process of importance for the maintenance of a continued discharge. Kohlrausch's results appear to exclude pigment or rod and cone movements as possible sources of P I. Its reactions to ether and asphyxia indicate a process of central origin (retinal synapses, cell bodies), perhaps akin to the slow changes recorded by Adrian and Buytendijk [1931] from a central structure (cf. also Birsch-Hirschfeld [1900] for histological changes in the retina after illumination).

The second process, P II, rises rapidly as the positive *b*-wave of the complex response, then falls fairly rapidly at high intensities, less rapidly at low intensities, and continues hidden by the first process under the *c*-wave of the complex action potential. It is the only process that can be detected at all intensities capable of giving a detectable response

and is of the same sign as the potentials recorded from non-vertebrate eyes. This component is associated with the production of impulses. P II is selectively affected by asphyxia and can also be removed with ether during prolonged narcotization. It thus reacts to ether and asphyxia as the negative potential recorded by Gasser and Graham [1932] in the spinal cord which they believe is connected with summation. The fall in the *b*-wave and the similar phenomenon observed by Adrian and Matthews in the frequency of the impulses probably represents, partly at least, a process of adaptation but is also dependent upon

The third process, P III: this is of negative sign and therefore by algebraical summation influences the amount of potential in the complex response. P III first appears as the *a*-wave of the composite potential, its further course is hidden, but by its return to zero at cessation of stimulation the positive off-effect is elicited as a release phenomenon. The off-effect has its counterpart in a renewed discharge through the optic nerve. This is held to imply that when P II is released from the negative P III it is being released from a process in some way concerned with the inhibition of impulses. By definition the off-effect is then a "post-inhibitory rebound." P III is the most persistent of the components of the retinal action potential. A potential wave of opposite sign to the one Gasser and Graham [1932] believed to be concerned with summation was also noted by them in the spinal cord. They suggest that it might be inhibitory in character. The eye performs several functions in which an inhibitory process should be useful [cf. Graham and Granit, 1931], but there is little reason to discuss them as long as this process presents a number of problems, accessible to experimental approach, which should first be solved.

Vision and retinal processes.

The previous pages should have made it evident that our knowledge of retinal physiology still is at the stage when even quite elementary facts have to be established about the nature of the processes concerned. Little can therefore be gained by theorizing extensively about the significance of this work for the subject of vision. It is not even possible at present to express the retinal action potential in terms of frequency of impulses. A hypothesis has been offered by Kohlrausch [1931], but we are clearly far from the stage at which elaboration of theories may prove fruitful.

But it is worth while to make one generalization: this is the necessity of realizing that the retina as a sense organ cannot be identified with

the rod-cone receptor system. The synaptic apparatus continuously modifies the primary response determined by the properties of the receptors. Special methods have to be developed to prove *in casu* that a measured function is due exclusively to, say, the photochemical processes in the receptors. Thanks largely to the work of Sherrington and his collaborators [see *e.g.* Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932] the physiology of the synaptic reactions has now been developed to a point when real significance may be derived from the fact that such reactions are present in the retina. A case in point is the question of area stimulated. In Part I it was pointed out that the action potential obtained with a small area and a high intensity differs, not only in amount, but also with regard to form and time relations, from the potential obtained when the area is large. Thus the reaction behind the potential has been organically changed to a low-intensity process by a diminution in area stimulated. Probably the number of active units also enters into the total effect in a manner determined by the electrical conditions in the tissue, but more important is the fact that they do so in a purely physiological way, making up a characteristic total reaction by way of processes of interaction at the synapses.

Considering the complications present already at the "sub-sensational stage," studied above, more work with the retina rather than with sensations would appear to be necessary for the establishment of a retinal physiology on a sufficiently broad and unprejudiced basis. Quantitative correlations between sensory phenomena studied through the medium of sensations and certain assumptions as to the nature of the photochemical mechanism of the receptors elaborated, for instance, by Hecht, can hardly give more information about the processes concerned than purely empirical equations.

Summary.

Of the three components of the retinal action potential only one, P II, can be shown to be associated with the discharge of impulses through the optic nerve. P III appears to be related to an inhibitory process. P I does not appear to be concerned with the discharge of impulses, or, if so, to a very small degree. These statements are summarized in greater detail on pp. 223 and 234.

Prof. Sherrington has placed at my disposal the facilities of this laboratory; for this and for his kind active interest in the work I am very grateful.

The electrical recording system was tried out in some preliminary experiments in the Physiological Institute at Helsingfors, where Prof. Y. Renqvist kindly provided me with assistance. I also wish to acknowledge the valuable cooperation of Dr J. C. Eccles and Dr G. L. Brown in some preliminary experiments with the oscillograph.

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EXPERIMENTS ON THE NATURE OF THE LABILE RÔLE OF SULPHUR IN METABOLISM.

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Previous experimental work by the writer [Wilson, 1925, 1926, 1931] appears to justify the conclusion that in the catabolism of protein the sulphur fraction is the first to be dealt with: it seems also that in the anabolic phase the sulphur takes a lead, although the evidence on this point is not so striking. The complex nature of the metabolic processes renders the separate analysis of these two phases difficult. They tend in the living cell under normal conditions to keep in equilibrium, and it is only when either anabolism or catabolism is predominant that the liability of the sulphur moiety is apparent. If, however, a metabolite containing sulphur is the mobile unit, the question arises at what phase of its metabolism is its liability exhibited. At present we know too little of the anabolic changes to attempt any investigation of this aspect of the problem. But we can attempt to find out at what stage in protein catabolism the sulphur fraction is most open to attack. The sole evidence, however, in favour of the preferential catabolism of sulphur lies in its early excretion in the urine for which several factors might be responsible. If it be assumed that ingested protein is built up into some complex after absorption and before it is metabolized there are three different phases in the catabolism and excretion of sulphur, any one or any combination of which might be responsible for its early elimination in the urine. In the first place if some complex is built up from the amino-acids derived from the food, in the process of catabolism some of the amino-acids may be released from the molecule before others. This question has not been investigated *in vivo* so far, but Abderhalden [1923] has shown that, in a tryptic digest of protein, tyrosine is split off more quickly than glutamic acid, while cystine is said to appear early in the digest. If this holds good *in vivo*, then the early excretion of sulphur is possibly due simply to the cystine being available for catabolism before the majority of the other amino-acids. In the second place, however, it is possible that free cystine is much more readily catabolized than the other amino-acids, hence the excretion of sulphur in the urine would tend to precede

the nitrogen. There is yet a third possibility, namely, that all the amino-acids are equally liable but that the sulphates are more quickly eliminated by the kidney than urea or ammonia. It is to be noted that the two latter possibilities might explain the early excretion of sulphur in catabolism but they would not explain the behaviour of sulphur in anabolism.

The first possibility, on the other hand, might be invoked to explain not only the preferential catabolism of sulphur but also its leading rôle in the anabolic phase, if it could be proved that in protein synthesis the sulphur is the first to be built up into the protein molecule.

Experiments were therefore planned to find out if either of the two latter hypotheses were true in regard to the catabolism of the sulphur fraction. The question of the preferential excretion by the kidney of sulphate over urea was first investigated. The experiments on this aspect of the problem were carried out on a female dog weighing 14 kg. The principle of the method was to feed ammonium sulphate and note the relative rate of excretion of sulphur and nitrogen. It is known that ammonium sulphate is changed in the body and excreted as an alkaline sulphate, while most of the ammonia is combined with CO_2 to form urea, although some of it may be excreted as such. The animal was kept in a metabolic cage during the course of the experiments and the urine was drawn off by catheter. In Exp. 1 (Table I) the animal was fasted for 3 days, and on the morning of the fourth day a solution of ammonium sulphate was given by mouth. Owing to the fact that a stomach tube could not be passed in this particular animal an exact record of the amount of the salt given was not obtained. This, however, does not invalidate the results as the object was to determine the Sulphur: Nitrogen ratio of the excess material

TABLE I.

Period	Diet	Total N g.	Total S g.	S : N	Excess N g.	Excess S g.	S : N of excess
			EXP. 1.				
I (6 hr.)	Fasting	0·753	0·034	1 : 22·14	—	—	—
II (6 hr.)	$(NH_4)_2SO_4$ administered	1·484	0·413	1 : 3·59	0·731	0·379	1 : 1·92
III (6 hr.)	Fasting	1·550	0·351	1 : 4·41	0·797	0·317	1 : 2·51
IV (12 hr.)	,,	1·121	0·208	1 : 5·38	—	0·070	—
IV (average per 6 hr.)	,,	0·560	0·104	1 : 5·38	—	—	—
V (6 hr.)	,,	0·460	0·035	1 : 13·15	—	—	—
			EXP. 2.				
I (24 hr.)	Basal 1	2·748	0·126	1 : 21·80	—	—	—
II (24 hr.)	Basal + 7 g. $(NH_4)_2SO_4$	4·048	1·264	1 : 3·20	1·300	1·138	1 : 1·14
III (24 hr.)	Basal	2·987	0·216	1 : 13·82	0·239	0·090	1 : 2·65

excreted over that in Period I. In order not to miss the early excretion of sulphur, the urine was collected in two periods of 6 hours following the administration of the salt.

In Exp. 2 (Table I) so as to induce the animal to take a larger quantity of the salt a standard diet was fed daily, and on the experimental day 7 g. of the salt were added. The urine in this experiment was collected over the 24 hours. The excess nitrogen and sulphur (hereafter N and S) and the S:N ratios were calculated, using the figures in Period I as basal values. The S : N ratios of the excess were then compared with that of the ingested salt. The standard diet employed had the following composition: 60 g. fat, 100 g. beef, 100 g. tapioca, 10 g. bone ash.

It will be noted that in Exp. 1 (Table I) the ratio of the excess is 1 : 1·93 in Period II and 1 : 2·5 in Period III. In the subsequent 12 hours the N output was below the basal value, while S was still being excreted. There is no indication of the preferential excretion of S as judged by the S:N ratio of the excess which does not even reach the theoretical for the salt ingested (1 : 0·87). The figures would have been more striking if Period V had been chosen, for the basal values as the N output is lower than in Period I, while the S is almost identical. In Exp. 2 (Table I), in a 24 hours' period, the same phenomenon was noted, and it may be concluded that the factor responsible for the early excretion of S in protein metabolism is not a selective excretion of sulphur.

A series of experiments were therefore planned to test the second possibility, namely, that cystine is more readily metabolized than the other amino-acids. If the food protein is absorbed and catabolized directly as a mixture of amino-acids, a comparison of the rate of elimination of S after cystine ingestion with the rate of elimination of N after a protein has been superimposed, should show whether cystine is catabolized more quickly than a mixture of S-free amino-acids. The principle of the method was similar to those previously recorded [Wilson, 1931]. The experiments were carried out on the writer as subject. A basal diet of bread and cheese, butter and jam was ingested until equilibrium was attained and the protein was superimposed for one day. The proteins employed were beef 250 g., gelatin 70 g. and beef 250 g. (Exps. 3, 4 and 5, Table II). The excess N and S outputs were calculated in the usual way, the basal values being obtained from the average of the first two and the last two days of the experimental period (14th, 15th, 30th and 31st Jan.). The percentage of the total excess excreted daily has been calculated for each experiment separately (the total excess excreted over the basal period for each experiment has been taken as 100 and its distribution over 2 to

TABLE II. (EXPS. 3, 4 and 5.)

Day	Diet	Total N g.	Total S g.	N above basal g.	Excess N excreted daily p.c.	S above basal g.	Excess S excreted daily p.c.
1	Basal	7·882	0·491	—	—	—	—
2	„ Exp. 3 Basal + 250 g.	7·627	0·480	—	—	—	—
3	Beef	10·301	0·739	2·303	70·5	0·239	87·8
4	Basal	8·960	0·533	0·962	29·4	0·033	12·2
5	„	7·784	0·464	—	—	—	—
6	„	7·952	0·454	—	—	—	—
7	„ Exp. 4 Basal + 70 g.	8·072	0·524	—	—	—	—
8	Gelatin	11·961	0·659	3·963	55·9	0·159	65·9
9	Basal	9·996	0·533	1·998	28·2	0·033	13·6
10	„	8·562	0·516	0·564	7·9	0·016	6·6
11	„	8·554	0·533	0·556	7·8	0·033	13·6
12	„	7·851	0·511	—	—	—	—
13	„ Exp. 5 Basal + 250 g.	8·285	0·533	—	—	—	—
14	Beef	11·544	0·791	3·546	46·4	0·291	63·6
15	Basal	10·301	0·607	2·303	30·1	0·107	23·4
16	„	9·293	0·527	1·295	16·9	0·027	5·9
17	„	8·201	0·527	0·203	2·6	0·027	5·9
18	„	8·282	0·505	0·284	3·7	0·005	1·0

5 days calculated). It should be noted that the assumption in these experiments is that ingested protein is absorbed and metabolized as a mixture of amino-acids. The main object of the experiments was simply to observe the rate of excretion of N alone. The S excretion, however, has been given in addition as it may also be compared to the S output after cystine ingestion. It will be seen in these three experiments that the percentage of S eliminated on the day of ingestion is higher than that of N. This again confirms what has been repeatedly observed, the preferential catabolism of S. It will be noted also that the excess N and S metabolized in Exp. 3 is relatively small, and consequently the percentage excreted on the day of superimposition is high. In Exp. 5 also with beef the excess metabolized is greater and is spread over 5 days. In Exp. 4 with gelatin the excess excreted is spread over 4 days. It will be seen that the percentage of both N and S excreted each day varied for the different experiments according to the number of days over which the catabolism of the ingested material was spread.

It remains now to compare those figures with the rate of excretion of S after cystine ingestion. A nitrogen-free diet (Exp. 6, Table III), similar to that previously employed by the writer, was taken, and on the sixth day 4 g. of cystine containing 1·060 g. S and 0·564 g. N were superimposed. The excess S was calculated by employing the S output on the

TABLE III.

Exp. 6.

Day	Diet	Total N g.	Total S g.	S : N	Excess S g.	Excess S excreted daily p.c.
1	Basal N-free	8·232	0·439	1 : 18·72	—	—
2	„ „	5·241	0·291	1 : 18·01	—	—
3	„ „	4·558	0·274	1 : 16·63	—	—
4	„ „	4·172	0·258	1 : 16·17	—	—
5	„ „	3·864	0·247	1 : 15·63	—	—
6	Basal + 4 g. cystine	4·816	0·835	1 : 5·76	0·588	47·53
7	Basal	4·480	0·555	1 : 8·07	0·308	24·89
8	„	4·064	0·500	1 : 8·12	0·253	20·45
9	„	4·648	0·335	1 : 13·87	0·088	7·11

Exp. 7.

Day	Diet	Total N g.	Total S g.	S : N	Excess N N g.	Excess N excreted daily p.c.	Excess S S g.	Excess S excreted daily p.c.
1	Basal N-free	4·065	0·348	1 : 11·68	—	—	—	—
2	„ „	3·654	0·268	1 : 13·63	—	—	—	—
3	Basal + 4 g. cystine + 250 g. beef	6·258	1·044	1 : 5·99	2·604	51·52	0·776	62·37
4	Basal	5·348	0·621	1 : 8·61	1·694	33·51	0·353	28·37
5	„	4·410	0·368	1 : 11·98	0·756	14·95	0·100	8·03
6	„	3·654	0·283	1 : 12·91	—	—	0·015	1·20

day before as a basal value. Over the 4 days 1·237 g. of excess S were excreted, and hence there was a small negative balance. The percentage of the total excreted on the day of superimposition was 47·53 p.c. and it fell to 7·11 p.c. by the fourth day. If these figures are compared with the percentage N excretion on each day after protein ingestion (Exps. 3, 4 and 5), it will be seen that they are less than those in Exps. 3 and 4 and approximately the same as those in Exp. 5. In Exp. 7 (Table III) 4 g. cystine and 250 g. beef were superimposed for 1 day on a similar N-free diet. It will be noted that of the total excess S excreted, namely 1·244 g. 62 p.c. has been eliminated on the first day and 1·2 p.c. on the fourth day. It should be observed that the total excess S is scarcely greater than in the previous experiment in spite of the fact that an extra 0·55 g. S was ingested as beef along with the cystine. Apparently the rate of elimination of the excess S has been accelerated by the metabolizing of the beef, as the percentage of the total excess S on the day of superimposition in Exp. 7 is 62·37 p.c., as opposed to only 47·53 p.c. in Exp. 5 with cystine alone, and this in spite of the fact that the total excess S excreted in each experiment is approximately the same, although an extra 0·55 g. S was ingested as beef in Exp. 7. It is probable, however, that this increased percentage of S excreted on the day of superimposition in Exp. 7 is due to the S of the beef being metabolized in advance. In Exp. 5 (Table II) with beef alone the percentage excretion on the day of superimposition

was 63·6 p.c. as compared with 62·37 p.c. in Exp. 7 where cystine and beef were ingested.

The evidence from these experiments does not support the hypothesis that cystine is more readily catabolized than any other amino-acids. This might be expected in the light of Lewis and Root's work [1922]. These investigators have shown that cystine is not metabolized unless its amino-group has been removed. In other words deamination precedes oxidation of the sulphur group. If the process of deamination is the same for all the amino-acids it would be expected that all those acids including cystine should be of equal lability. In view of the evidence which shows that the sulphur moiety is possibly the mobile unit both in anabolism and catabolism and further that, as far as catabolism is concerned, the labile phase is not in the final process of demolition of the amino-acid, it would appear that a final solution of both aspects of its metabolism will be found in its position in the protein molecule or even the living protoplasmic unit. In the breakdown of protein the sulphur might be split off first, and hence it would be available for catabolism and early excretion in the urine. The preferential retention of this element might correspondingly be due to the unit containing sulphur being taken up to form perhaps not the nucleus but possibly the keystone of the protein molecule. The sulphur moiety would hence both confer and condition the stability of the biological unit. Its lability might, therefore, be due not to any inherent property of, say, cystine but rather to the position it takes in relation to the other constituents of the protein molecule. In this connection some observations of Hopkins [1930] are of interest. He showed that one of the features of denaturation of egg albumin is the appearance of the thiol group as shown by the nitro-prusside reaction. This opens up the possibility that, in the catabolism of protein, some molecular change may take place whereby the sulphur fraction is more readily oxidized or split off from the protein molecule. A sulphur grouping, at all events, appears to be in a readily accessible position in the protein molecule.

Summary.

1. A series of experiments were carried out in order to elucidate at what phase in the metabolism of protein the liability of the sulphur moiety is apparent.

2. Three possible phases are discussed: (1) that the early excretion of sulphur in the urine is due to the kidney excreting sulphates more

readily than urea or ammonia; (2) that cystine is more open to attack than the other amino-acids; (3) that cystine or some unit containing sulphur occupies a key position in the protein molecule whereby it is the first to be split off in catabolism and the first to be retained in anabolism.

3. The experimental results do not favour the view that the first two possibilities can explain the phenomena. The last hypothesis is held to explain most satisfactorily the labile rôle of sulphur in both phases of metabolism.

I desire to acknowledge my indebtedness to Prof. Cathcart for much helpful advice and criticism during the progress of the work.

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THE ANTAGONISTIC EFFECT OF ALCOHOL ON PITUITRIN HYPERGLYCÆMIA.

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In a series of experiments on normal human subjects Edkins and Murray [1931] showed that the ingestion of alcohol with glucose decreased the degree and duration of the hyperglycæmia. Hunt [1930], in diabetic patients, showed that alcohol definitely decreased hyperglycæmia. In the experiments on normal subjects the degree of hyperglycæmia when alcohol and sugar were taken together seemed to be related to the amount of diuresis. When the hyperglycæmia was small the diuresis was big and *vice versa*. As a result of this relationship a suggestion was put forward that a possible explanation might be, that alcohol inhibits the secretion of the posterior lobe of the pituitary gland, or in some way prevents the development in the body of its ordinary action. Injections of an extract of this lobe ("pituitrin") are known to produce hyperglycæmia, and also antidiuresis or diuresis according to the conditions of experiment. In a more recent paper Murray [1932] has shown that the ingestion of alcohol antagonized the effect of pituitrin injection on water diuresis in normal human subjects; and in anæsthetized cats diminished the diuretic response to pituitrin. The experiments to be described here were designed to test the effect of alcohol on the hyperglycæmic response to pituitrin injections.

Cats, kept on a generous diet of meat and milk for at least a week, were deprived of food for 18 hours before the experiments. They were given intra-peritoneal injections of amytal with no preliminary anæsthesia. The carotid artery on one side was exposed for the taking of blood samples, the puncture in the artery being clipped without occlusion of the artery. A tube was tied into the œsophagus for the administration of liquids. The hyperglycæmic effect of pituitrin in cats has been systematically investigated by Clark [1927], and shown to be due to release of glycogen from the liver. It was not possible to compare the effect of pituitrin in the same animal before and after alcohol, because the liver glycogen continually decreases in animals under amytal, and also because one response

to pituitrin might diminish the effect of a second injection. This difficulty was surmounted to a certain extent by making a large number of experiments; these can be divided into four groups:

(1) Hyperglycæmia due to a single injection of pituitrin.

(2) Hyperglycæmia due to a single injection of pituitrin after giving alcohol.

(3) Hyperglycæmia due to two successive injections of pituitrin.

(4) Hyperglycæmia due to one injection of pituitrin and then a second injection, alcohol being given between the injections.

Blood samples were taken and the sugar estimated until the blood sugar was constant. The estimations were made by the method of Hagedorn and Jensen. The dose of pituitrin, 0·5–0·75 c.c. of Parke-Davis pituitrin (1 c.c. = 20 units), was injected into the saphena vein. When alcohol was given, the amount was 20 c.c. of a 20 p.c. solution, this was introduced into the stomach through the tube in the œsophagus. The results of the injections of pituitrin on the blood sugar under the four different conditions set out above are shown in Table I.

TABLE I. The rise in blood sugar in mg./100 c.c. caused by an injection of 0·5–0·75 c.c. of pituitrin.

No. of Exp.	Pituitrin, first dose	Pituitrin, first dose after alcohol	Pituitrin, second dose	Pituitrin, second dose after alcohol
1	66	—	—	—
7	180	—	—	—
2	—	−30	—	—
3	—	31	—	—
4	—	4	—	—
5	—	19	—	—
29	—	33	—	−20
6	76	—	—	19
10	99	—	—	5
12	49	—	—	4
13	44	—	—	11
16	60	—	—	0
27	55	—	—	11
22	—	—	—	−12
8	102	—	70	—
15	86	—	145	—
36	78	—	85	—
35	123	—	65	—
33	86	—	20	—
32	87	—	40	—
Average	85	11	71	2

From these results it can be seen that after giving alcohol an injection of pituitrin, whether a first or second dose, does not evoke a characteristic hyperglycæmic effect. The way in which alcohol suppresses the rise of

blood sugar is not clear. One possibility is that the alcohol, during absorption, extracted something from the intestinal wall which was carried into the blood and prevented the action of pituitrin. To test this point in a number of experiments the alcohol was given intravenously. In the first few experiments of this kind small doses of alcohol were given, and in these a considerable rise in blood sugar was obtained, so that it seemed that alcohol acted differently according to the mode of administration. In later experiments where the alcohol in the blood was estimated it was obvious that these doses had been too small. When the alcohol was given by stomach tube the alcohol in the blood often attained the value 125–150 mg./100 c.c., a figure obtainable in man by the drinking of about a litre of 8 p.c. alcohol. To get this concentration by injection, almost as much was required as by mouth. This is probably because the high concentration of alcohol in the blood in the first few minutes after the injection leads to a very rapid penetration into the tissues, and also because, when given by mouth, the absorption is rapid and complete and the rate of oxidation very slow. The suitable injection dose was about 15 c.c. of 20 p.c. alcohol in saline. In the experiments where this large dose was given no hyperglycæmia was obtained after injecting pituitrin. Hence the determining factor is definitely the concentration of alcohol in the blood. The results in Table II, relating concentration of alcohol and rise of sugar

TABLE II. Relationship between concentration of alcohol in blood and the rise in blood sugar level caused by pituitrin injection.

No. of Exp.	Conc. of alcohol in blood mg./100 c.c.	Rise in blood sugar mg./100 c.c. after pituitrin, first dose	Rise in blood sugar mg./100 c.c. after pituitrin, second dose	Remarks
40	196	10	−11	Cat 3·3 kg. 16 c.c. 20 p.c. alcohol intravenously
42	145	16	18	Cat 3·3 kg. 15 c.c. 20 p.c. alcohol intravenously
41	79	128	35	Cat 3·7 kg. 14 c.c. 20 p.c. alcohol intravenously
39a	46	86	—	Cat 2·75 kg. 7 c.c. 10 p.c. alcohol intravenously
39b	93	—	40	Further dose of 10 c.c. 20 p.c. alcohol intravenously
38	154	24	—	Cat 3·0 kg. 20 c.c. of 20 p.c. alcohol by mouth

level in the blood in response to pituitrin injection show this to be so. It seems that alcohol, then, diminishes and checks hyperglycæmia. Small doses given to normal human beings lower the blood sugar slightly

[Edkins and Murray, 1931]. In the Allen treatment of diabetes, whisky was often given because it diminished the glycosuria. The points arise then as to how alcohol reduces and prevents hyperglycæmia, and what is the fate of the sugar. Experiments are being carried out to test whether alcohol prevents adrenaline hyperglycæmia, and also to determine the effect of administration of alcohol on the glycogen content of the liver.

Summary.

Alcohol administered to cats by mouth or intravenously, so that a concentration of over 100 mg./100 c.c. of blood is attained, prevents the rise of blood sugar which normally results from the injection of pituitrin.

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OBSERVATIONS ON SENSATION.

The sensory functions of the skin for touch and pain.

By DAVID WATERSTON

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Research upon cutaneous sensation in Man during recent years has been directed largely to the study of the results which follow immediately upon experimental division of one or more cutaneous nerves in the observer's own person, and of the gradual recovery of the different forms of sensation—touch, pain, pressure, temperature. These phenomena are puzzling, and different observers are not agreed upon their significance and the interpretation to be put upon them, or on the light which they throw upon the nature of the normal sensory apparatus.

H. Head [1905], from the study of the results of section of two cutaneous nerves in his own arm, put forward his widely quoted theory of the presence of a dual sensory mechanism, each subserving certain forms of sensation and having distinctive characters, generally expressed as a "protopathic" or crude "defensive" sensibility, which serves for the perception of pain and of extreme degrees of temperature, tending to radiate, and an "epicritic" sensibility, presumed to be phylogenetically a later acquisition, which includes what is termed tactile sensation and the discrimination between moderate degrees of temperature.

Head's observations and the conclusions which he drew from them have recently been criticized drastically by Sharpey-Schafer [1929], from the results following nerve section and nerve crushing on his own person. He reached the conclusion that Head's "protopathic" nerve fibres are nothing more than the ordinary "pain fibres" reacting in a peculiar manner from the altered conditions produced by the operation on the nerve trunk and by the growth of the axis cylinders.

The difficulties experienced in interpreting the results of such experiments are without doubt due to our ignorance of the normal mechanism of the production of the different forms of sensation. This is especially

the case in regard to pain, but it holds also for tactile sensation, for temperature sensation and for pressure. Touch is spoken of as having its receptors in the corium, pain as arising from nerves of the epidermis. (In view of the facts described here these statements are erroneous.)

The following observations are a continuation of those on touch and temperature which have been already published [Waterston, 1923]. The training undergone preliminary to these former observations and the experience gained in carrying them out have been of great value. Those who have attempted observations on sensation are agreed that a long preliminary training is essential if accurate and reliable results are to be obtained.

The sensations dealt with here are those of touch ("tactile sensation") and of cutaneous pain, the first the sensation excited from the surface of the skin by light touch with a fine bristle or brush drawn lightly over the surface or applied to individual points.

Pressure is not a cutaneous sensation, it is not elicited by pressure applied to the surface of a fold of skin and subcutaneous tissue, but it is excited by pressure on the surface if sufficiently firm to affect deeper tissues such as muscle or periosteum, etc., and its consideration may be excluded here. Cutaneous pain was tested by the prick of a sharp needle.

It has been shown by many writers that the nerves of the epidermis arise from medullated fibres which form the subpapillary plexus and they lose their medullary sheath when they enter the epidermis, run near one another almost vertically and can be traced to the uppermost layers of the epidermis. Two main varieties of fibres have been found. Some are thick with few varicosities in the deeper layers, and in their course through the stratum mucosum they give off a number of lateral branches which end in small knobs. Other extremely thin fibres are present also, of such fineness that they can be detected only with the highest powers of magnification and can hardly be distinguished from nerve fibrils. These also give off lateral branches [Stöhr, 1928].

Some time ago I put forward evidence [Waterston, 1931] to support the view that the receptor for tactile sensation is in the deeper layers of the epidermis, that some of the cells of that layer are the receptors for touch and that the nerves of the epidermis are tactile nerves. The evidence upon which this view was based lay in the facts that the anatomical arrangement of the parts was appropriate to such a function, that the sensation of touch cannot be elicited from a skin surface denuded of epidermis and that the stimulus adequate to elicit the sensation of touch

in the normal skin is so slight as to render it almost impossible that the deeper organs of the corium (Pacinian bodies, Meisner corpuscles, etc.) can be the receptors for touch as is generally stated. The investigation has now been carried further and the conclusion reached that not only are the nerves of the epidermis the nerves of touch, but also that they are nerves of that specific sensation alone and that they do not convey impulses which give rise to pain. The pain mechanism is separate and distinct and lies in a deeper layer of the skin. It was pointed out in a former paper [Waterston, 1931] that, in the living person, epidermis could be cut away through its deeper layers (stratum mucosum) without eliciting the slightest pain but only a sensation of touch, even though, as might be inferred, nerve fibres were divided in making the cut. To complete the evidence it was felt that it was necessary to show that this actually occurred, and search was made to discover if this were so. The method employed was to shave off a portion of cuticle as deeply as to the level of the stratum mucosum and deeply to the stratum granulosum. The colour of the intact skin is modified by the granules of the stratum granulosum, and when that layer is removed the exposed surface is of a bright pink colour. It was found necessary to remove the portion of skin to be examined by a single cut to the required depth rather than by shaving it away in successive slices, for while thin sections can be cut as deeply as to the stratum granulosum, they cannot be obtained from the normal skin at a deeper level for the cuticle comes away in small fragments. Section of the skin as described were made in several individuals and in different regions with uniform results. It can be done, however, only in regions where the epidermis is of sufficient thickness.

In the forearm, for example, the epidermis is so thin—1/20 or 1/30 mm. in thickness—that it cannot be shaved away without cutting into the corium and producing pain.

The front and sides of the terminal part of the fingers provide the most suitable site, for there the epithelium is sufficiently thick to allow slices to be shaved off without cutting into the corium.

The areas where sections were made were tested for their sensitiveness to different forms of stimulation, and were responsive to touch, to heat and to cold as well as to pain while the skin was intact.

During the removal of the epidermis the only sensation elicited was that of touch; no pain at all was felt.

Examination of the surface exposed by removal of the greater part of the epidermis showed that the sensation of touch could be elicited from it by the same form of stimulus as before. The sensation, however,

was not entirely the same, it was less definite, more of a prickly nature. Description of the difference is not easy, but the experience of the different persons examined was the same, and the sensation was described as not the same as from the untouched skin.

Possibly all the tactile nerve terminals had not been removed, and some remained behind in the deeper layer of the epidermis, but the explanation of the difference in sensation is probably to be found in the fact that on the denuded area the contact stimulus was applied directly to the divided ends of the tactile nerves and not to the "receptors" with which they are normally in contact.

The small size of the denuded area rendered it unsuitable for the precise investigation of temperature perception on its surface, and it was impossible to identify on the denuded surface the exact position of any touch or temperature spot which had been detected on the epidermal surface while it was intact. So far as could be made out, both cold and warmth could be felt on it, but warmth very quickly passed into pain which obscured the former sensation.

The portions of epidermis removed were stained with gold chloride and lemon juice (Ranvier's method), embedded in paraffin and cut usually vertical to the surface, but in some specimens parallel to it. Microscopical examination of the sections showed that the cut had gone to the required depth, the portions removed including the stratum granulosum and a considerable thickness of the stratum mucosum, which it had traversed superficial to the tips of the papillæ of the corium.

Excellent histological pictures were afforded of the layers of the stratum granulosum, whose granules show up well with this method (gold chloride and acid) of staining.

Since many investigators have demonstrated the presence of fine nerve fibres in epidermis, it is not surprising that we have found nerve fibres and their terminals present in the sections of the portions of skin which we have removed. The fibres are fine, their course is tortuous and they were found only in short lengths in any one preparation. They appeared as short thin rods, lying between the epithelial cells, deeply stained of a dark purplish colour, in some cases slightly varicose, in others with small branches (Fig. 1). They were found in the stratum mucosum and extended as far as to the deep surface of the stratum granulosum. The appearances which they presented were similar to those figured by Ranvier [1880] and to Kadanoff's figures [1924] of nerve fibres among the epidermal cells of the pig's snout, though they were much fewer in number in our preparations.

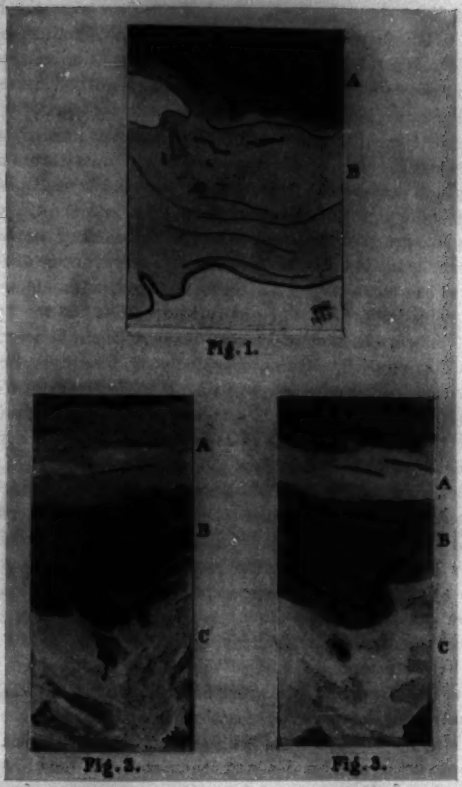

Fig. 1. Afferent nerve fibres and a terminal in the stratum mucosum of a piece of removed epidermis. Gold chloride and lemon juice. Camera lucida. × 340. (A) Stratum granulosum. (B) Stratum mucosum.

Fig. 2. Afferent nerve terminal in the stratum mucosum of a portion of removed epidermis, showing its position close to the stratum granulosum. The varicose nerve fibre ends in an arborescent figure on the surface of a cell of the stratum mucosum. Gold chloride and lemon juice. × 340. Camera lucida drawing, with the detail completed with 1/12 in oil-immersion lens and Swift "C" ocular. × 600. (Section slide W 3, section 3.) (A) Stratum lucidum. (B) Stratum granulosum. (C) Stratum mucosum.

Fig. 3. Next adjacent section of the same terminal, showing the fibrils of the arborescent figure. (Section slide W 3, section 2.)

More distinct than the nerve fibres themselves were the loops and figures in which they terminate. Terminals of this nature have been figured by Kadanoff, and our preparations showed similar appearances. They form arborescent figures of fine threads, slightly varicose, on the surface of epithelial cells in the stratum mucosum (Figs. 2, 3), and they constitute one type of termination of afferent nerves in relation to their receptors, which in this case are some of the cells of the stratum mucosum.

The nerve fibres and the terminals are exceedingly fine and are to be found only with high powers of magnification, and the use of an oil-immersion lens is usually necessary for their satisfactory demonstration.

This demonstration of the presence of nerve fibres and of their terminals in the tissue which had been removed, without causing any pain, completes the evidence necessary to establish the theory which I have put forward, viz. that the nerves of the epithelium are the nerves of light tactile sensation, and that they do not under ordinary conditions convey impulses which give rise to pain. If so severe a form of stimulation as division of these nerve fibres does not give rise to pain, it seems impossible that any other form of stimulus can do so. We should, in other words, regard the epidermis as the receptor tissue for the sensation of touch, with its "receptors" in the deeper layer of epidermic cells and its specific nerve fibres which, like other nerves of special sense, do not give rise to pain even when they are divided. The sense of touch follows Johannes Müller's law, and impulses transmitted by its fibres give rise to no other sensation than the specific special sensation. As the optic nerve can be divided without causing pain, similarly cutaneous tactile nerves can be also without giving rise to pain.

If the evidence now obtained of this theory be accepted it will be necessary to abandon two views which are widely quoted, viz. that nerve fibres of epithelium subserve the sensation of pain, and that the Pacinian bodies or Meisner corpuscles are the receptors for light touch. Both of these theories are now untenable, and other functions must be sought for these and other similar organs of the corium.

Sensibility to superficial pain on the other hand is subserved by the corium. With the twofold structure of the skin, epidermis and dermis, there is a division of sensory function, the epidermis the organ of touch, the corium that of superficial pain.

The conclusion reached here, that the nerves of touch in Man do not convey pain-producing impulses is in harmony with the current physiological view that alterations in the frequency of impulses in afferent nerve fibres modify the intensity, but have no influence whatever on the

quality of the sensation, and confirm and amplify the conclusion reached by Adrian [1932], from investigation of the nerve impulses in cutaneous nerves in the frog and guinea-pig, in which he states that in those animals "the receptors for touch are supplied by the larger medullated fibres and that in some at least of these fibres no messages can give rise to pain in the normal animal."

Miss M. H. Kidston, was responsible for the preparation of the sections and making the drawings from them, and I am greatly indebted to her for the careful work which she has done in this connection.

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SINGLE-SHOCK EXCITATION AND INHIBITION OF CONTRALATERAL EXTENSION IN THE SPINAL CAT.

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An induction shock given alone and unrepeated is a relatively ineffective stimulus for the crossed extensor reflex. Yet even in the spinal preparation (cat) we have found, provided the spinal transection has been performed aseptically some days previously, such a stimulus does in the majority of "chronic" spinal preparations suffice to evoke a contraction in the contralateral extensor muscle.

Method.

Spinal transections were performed at the level of the first lumbar or last thoracic segment under deep anæsthesia and with full precautions against sepsis. Post-operative care consisted in regular emptying of the bladder and expression of the fæces by manual pressure, and, particularly when the animal was incontinent, of cleaning and drying the hind-quarters. If care is taken, spinal cats can be kept in a healthy condition for almost any desired period. In the experiments recorded in this paper twenty-six chronic spinal cats were used. The operative wound healed by first intention except for two instances. The time elapsing between spinal transection and myographic examination varied between a few days and 3 months.

Preliminary to myographic examination, the animals were decerebrated by the trephine method under full ether anæsthesia, all muscles of the hind-limbs except the one under examination, *M. soleus*, were paralysed by nerve or tendon section, and the preparation rigidly fixed for isometric recording by means of drills through both heads of the bone caught securely by clamps affixed to the iron top of a heavy table. The myograph used was the "frictionless" pattern of the isometric mirror myograph arranged for simultaneous shadow recording of a galvano-

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meter string, signals, etc., as described by Sherrington [1930]. In some experiments the myograph was the steel torsion-strip shadow recording type. Muscle action currents were led by Ag : AgCl pin-electrodes to a Cambridge string galvanometer. By means of a Lucas pendulum, electromagnetically released from the falling-plate camera, single break-shocks from two independent coreless induction coils were applied to the nerves through glass-shielded electrodes. For studying inhibition, the knock-down keys were wired so that stimuli to an ipsilateral and a contralateral nerve could be delivered at all intervals apart up to 465σ. The order of their fall was reversible.

Latent periods of single-shock reflexes were recorded by a derivation of the primary current of the exciting inductorium passing through the same galvanometer string that received the muscle action currents. The derivation shunting the galvanometer string was given a resistance of 10,000 ohms to avoid impairing the sensitivity of the string. The latent period was then directly measured from the string signal to the beginning of the action current.

Results.

The characteristics of the single-shock crossed extensor reflex in spinal chronic animals to which particular attention has been paid include (*a*) latency, (*b*) magnitude, (*c*) duration, and (*d*) behaviour to single-shock ipsilateral inhibition.

(*a*) *Latent period.*

The latency of the single-shock crossed extensor reflex of *soleus* throughout a series of chronic spinal cats varied considerably from animal to animal. The latent period measured by a derivation of the primary circuit of the induction coil through the galvanometer string used as a signal typically lies within the range of 20–35σ for the shortest latency recorded from each preparation. It may, however, reach the very low figure of 8·7σ; and the shortest latent period recorded from some preparations was as long as 50–60σ even when the reflex was of good magnitude.

The shortest latent period encountered (8·7σ) was from a preparation in which the spinal cord had been transected 84 days previously, and had throughout exhibited a marked extensor activity. The brevity of latency was consistent with the behaviour to inhibition, for it was necessary to allow a single ipsilateral break-shock a definite lead on the excitatory stimulus in order completely to suppress reflex contraction. The single-shock crossed extensor reflex may, therefore, exceptionally possess a latent period of the same order as the knee-jerk or its ipsilateral

inhibition [Jolly, 1910; Ballif, Fulton and Liddell, 1925] and the spinal flexion-reflex [Eccles and Sherrington, 1931*a*].

The spinal crossed extensor response appears to have a latency no longer on the average than the latent period of the crossed extensor reflex in the decerebrate preparation from a single stimulus [Forbes and Cattell, 1924; Pi-Suñer and Fulton, 1929] or even from tetanic stimulation [Fulton, 1926, p. 303; Eccles and Granit, 1929; Ranson, Hinsey and Taylor, 1929].

A period of 60–100σ, of which not more than a tenth part can be occupied by peripheral conduction, sometimes elapses between the single stimulus and the onset of contraction in the contralateral *soleus*. A latent period of this length calls to mind rebound contraction [Sherrington, 1913], but the frequent brief latencies encountered are a guarantee that the crossed response is a direct excitation. A long latent period is sometimes attached to a vigorous and highly repetitive central discharge. If the long latent period were required for summation sufficient to bring the first motoneurones to the point of discharge, it is unlikely that more than a feeble and single discharge would result. Impulses may reach the anterior horn cells early in the latent period in amounts adequate for discharge, but are suppressed by inhibition. In either case, impulses must arrive at the anterior horn cells as late as $\frac{1}{10}$ second after application of the stimulus. The temporal dispersion of the afferent volley traversing the spinal cord must therefore be great. The extreme variability in latency characteristic of this reflex is evidence that latency is conditioned by some functional and variable (? summative) process and not by fixed delays at synapses, or slower fibre conduction, or some other feature of reflex conduction depending on the nature of the path across the spinal cord.

(*b*) *Magnitude of reflex response.*

A small proportion of animals examined were totally impervious to a single contralateral excitatory afferent volley, an experience shared by those who have studied spinal and decerebrate preparations [Sherrington, 1910; Forbes and Cattell, 1924; Pi-Suñer and Fulton, 1929]. In other preparations the reflex was irregular and fugitive. There remained, however, a substantial percentage of animals in which the reflex was both strong and regular, though even in favourable preparations considerable variation in the reflexes from identical stimuli has been the rule. The maximum tension developed in *soleus* by a single shock to a contralateral afferent nerve for the individual chronic spinal animal ranges between 50 and 600 g.

Particular interest attaches to the single-shock form of a given reflex because of the opportunity it affords for judging the character of the central discharge. Thus, a single, break-shock, ipsilateral flexion reflex of *tibialis anticus* may exceed in tension the maximal motor twitch of the same muscle [Sherrington, 1921]. This may hold even though a maximal tetanic reflex from the same afferent nerve engages only a fraction of the total motor units making up the muscle [Cooper, Denny-Brown and Sherrington, 1926]. The single-shock crossed extensor spinal reflex can, like the ipsilateral flexion reflex, exceed in contraction tension the maximal twitch of the same muscle recorded immediately afterwards at

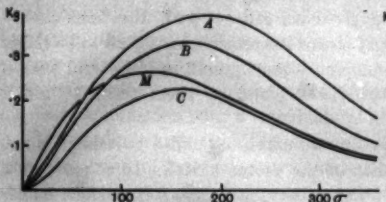

Fig. 1.

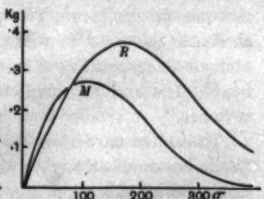

Fig. 2.

Fig. 1. Comparison of single-shock crossed extensor reflexes with the maximal motor twitch (*M*). Chronic spinal cat, 28 days. Deafferented soleus muscle. *A*, *B*, *C*, reflexes at 13, 15, 17 cm. coil distance; *M* at 20 cm. from uncut motor nerve. Weight of muscle 1·77 g.

Fig. 2. Same. 38-day cat, undeafferented muscle. *M* is motor twitch at 27 cm. (max.), *R* is reflex at 17 cm.

the same initial tension, etc. Experiments of this type are shown by tracings in Figs. 1 and 2. Such superiority is evidence that the central discharge cannot consist merely of a single efferent volley even if, which is unlikely, all motor units composing *soleus* are involved. On the contrary, some motor units exhibit more than one centrifugal volley and by the resulting peripheral summation enable the tension of the reflex to be greater than a motor twitch.

(*c*) *Duration.*

When, as is usually true of the single-shock crossed extensor reflex, the tension developed does not attain that of the motor twitch, there is still another feature of the myogram which indicates the character of the central discharge, namely, the duration of contraction. The shortest duration of the single-shock reflex contraction was 81·7σ, as measured by

the "contraction time," *i.e.* the interval from the first sign of electrical response to the point of maximum tension [Cooper and Eccles, 1930]. Contraction times of the order of the motor twitch, *i.e.* 80 to 120σ, do occur, but are not the rule. In general the reflex contraction time falls within the range 140–200σ. A variant showing after-discharge of proprioceptive origin is a regular twitch-like contraction to which is appended at some point in relaxation a long and sometimes clonic contraction. Prolongation of duration may mean that the muscle receives a single, but temporally dispersed, efferent volley; since enduring contraction from asynchronous firing occurs at the expense of tension, the combination of ample contraction with long duration points to repetitive central discharge. The single-shock reflexes with durations closely similar to the motor twitch, and also of less tension, are taken to be single volley reflexes such as were commonly encountered in ipsilateral flexion by Eccles and Sherrington [1931*a*] and by them termed "reflex twitches."

The action current of these crossed extensor "reflex twitches" is not dissimilar qualitatively to that of the motor twitch. It consists of a single initial excursion not followed by the delayed excursions which accompany more prolonged reflexes. In the latter the string galvanometer typically shows activity for a period of many sigmata, and the initiation of mechanical response is often accompanied by only slightly greater excursion, which is always far smaller than a motor twitch action current. Judging from the action current, this reflex is more synchronized in the spinal preparation than in the decerebrate as described by Forbes and Cattell [1924].

Pi-Suñer and Fulton [1929] have recorded decerebrate single-shock crossed extensor reflexes in deafferented vastocrureus with a duration three or four times the motor twitch duration. This degree of prolongation seems never to be approached by the spinal reflex of *soleus* whether normal or deafferented. Observations of Sherrington [1910] and Graham Brown [1911] on tetanic crossed reflexes have shown that after-discharge is curtailed in the spinal form. The crossed extensor "reflex twitch" seems peculiar to the spinal animal. Repetitive central discharge is not an invariable feature of the single-shock spinal crossed reflex. The highly repetitive central discharge of decerebrate crossed extensor reflexes must, therefore, receive explanation on grounds other than features of reflex conduction across the cord as seen in the basic spinal mechanism.

(*d*) *Behaviour to single-shock inhibition.*

The elicitation of a reflex response in an extensor muscle by a single unrepeated contralateral stimulus permits observations on the interaction of inhibition and excitation under some experimental conditions hitherto untried.

The reflex has certain advantages for the investigation of the time relations of the central inhibitory state: first, the central effects of both excitatory and inhibitory stimuli are uncomplicated by summation with inhibition or excitation from brain nuclei; second, the opportunity for repercussion of inhibitory impulses within the nervous system is limited; third, the inhibitory path, being ipsilateral, is short; fourth, proprioceptive excitation at initial tensions used is, if present, subliminal. For these reasons the central inhibitory state may be thought of as running its course relatively uninfluenced by excitatory impulses until brought into algebraic summation with a contralateral afferent volley.

By sampling the excitability to a single contralateral break-shock (*E*) at a succession of intervals before and after delivery of a single-shock inhibitory stimulus (*I*) to an ipsilateral nerve, it is possible to follow the changes in size of the reflex response corresponding with the interval of separation of the two shocks. Examples of such experiments are given in Figs. 3 and 4.

In the graphs the ordinates express the reflex contraction tension developed when the contralateral, *i.e.* excitatory, stimulus follows (*I–E* interval, + values to right) and precedes (*E–I* interval, – values to left) an ipsilateral inhibitory shock. The intervals *I–E* and *E–I* are shown by distance on the abscissa line. Owing to the variability in magnitude of the control *E* response our practice was to record alternately with the inhibited reflex an uninhibited control reflex. These are entered over their fellow inhibited reflex. The vertical distance between the average of the controls and the line joining the inhibited reflex points is an index of inhibition existing at any given *E–I* or *I–E* interval.

In the first limb of the curves the excitatory preceded the inhibitory stimulus because, owing to latency, the inhibitory shock may fall after the excitatory and yet be effective [Sherrington, 1925]. The first critical point is the *E–I* interval at which the central process underlying the contraction ascent has just been completed before the *I* volley reaches the centre or develops its effect. As the *E* stimulus is allowed less and less lead over *I*, the reflex contraction suffers progressively a loss of tension. A second critical interval is that at which an *I* stimulus falls just sufficiently early to block all reflex discharge. The stimulus, maximal in strength, is also just optimal in point of time relation to the excitatory stimulus. As the *I* shock is brought nearer to and made to

precede the *E* stimulus there is usually a steadily maintained deep inhibition, after which the value of the contraction response commences to rise, indicating the omission of the earlier excited motoneurones from inhibition. Now if the *E* reflex be initiated at longer and longer intervals after *I*, it steadily increases in tension. Often just before normal size is gained the curve of recovery flattens.

The position of the four points of inflection varies greatly in different experiments. The inhibition from a single shock may persist as long as

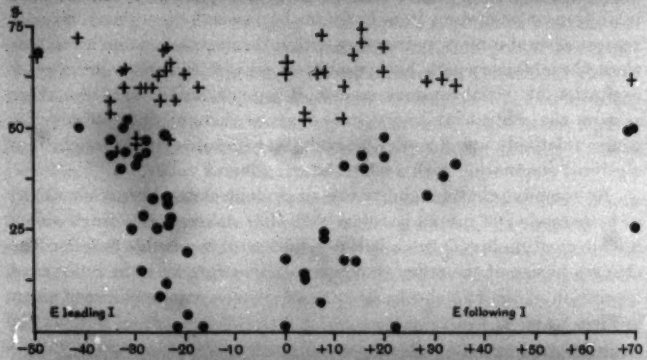

Fig. 3. Inhibition of the single-shock crossed extensor reflex by a single ipsilateral stimuli falling at intervals preceding (+) and following (−) the *E* stimulus. The ordinates give contraction tension. Zero on the abscissa line represents the application of the inhibitory stimulus and the position of a dot on the abscissa scale gives the time of application of the excitatory stimulus. A dot is an inhibited reflex, a cross an uninhibited control reflex recorded immediately after one of the inhibited reflexes beneath it. *E* stimulus 20 cm. coil distance, *I* stimulus 24 cm. Average latency of control reflexes is 29·5σ.

465σ, the longest interval open to exploration with the Lucas pendulum; or more rarely, it may run through its course in a tenth of this time. Curves of almost all intermediate grades have been mapped out, but usually the recovery of excitability has been slow. A long enduring inhibitory after-action may represent an equally long enduring central inhibitory state, *per se*, or merely a rapidly effected destruction of some facilitative background essential to the reflex, which is but slowly reconstituted [Sherrington, 1929]. Against the latter possibility is that typically long persisting inhibitory after-discharge has been demonstrated after deafferentation of the muscle.

In addition to earlier observations several recent studies [Ballif, Fulton and Liddell, 1925; Samojloff and Kisselew, 1927; Eccles and Sherrington, 1931*b*, 1931*c*; Bremer, 1931] are in accord that a single inhibitory volley has characteristically a prolonged after-action when excitatory destruction or masking by inhibition is not an obvious factor. The same conclusion can be extended to single-shock inhibition

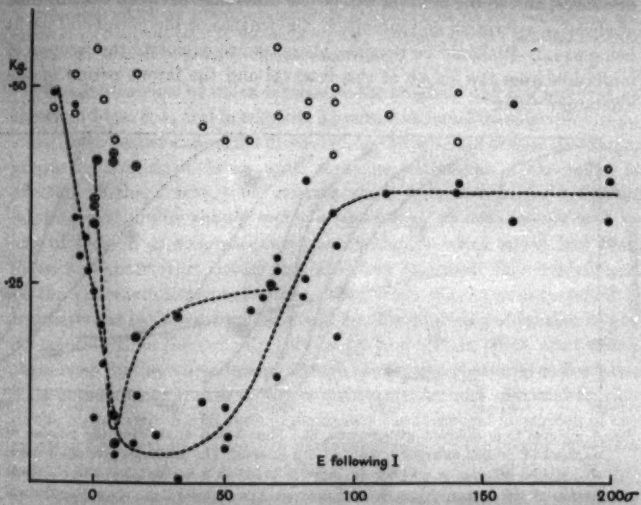

Fig. 4. Same as Fig. 3. Circles represent tension of control reflexes paired with underlying dot. *E* is 20, *I* is 25 cm. coil distance. Crossed and dotted circles are another series from the same preparation with *E* at 20 and *I* at 25 cm.

of this single-shock crossed extensor reflex. This type of inhibition is not, however, necessarily prolonged. There are some instances of brief-lasting inhibition in our results. The briefest inhibition recorded by us (Fig. 3) reached an optimum in about 15σ after the first sign of inhibition was manifest; after a maximal plateau of 10σ the decline of inhibition was largely over in another 25σ. Inhibition on some motoneurones was as short as 25σ, and 50σ is the longest time for which inhibition of any motoneurone can have persisted. In one series of observations in Fig. 4 the cycle is complete within 100σ. But since these preparations were undeafferented the factor determining the rate of disappearance of

inhibition may still be the amount of subliminal excitation which the inhibitory volley meets.

The greatest *E–I* interval for occurrence of complete inhibition is determined by the relative latency of *E* and *I* reflexes. The earliest inhibitory impulses must arrive not later than the time at which the excitatory impulses reach the motoneurones in quantities adequate for discharge, and so the interval will be the difference between central time of effective excitation and inhibition, the lengths of the peripheral paths being equal. It should be possible, therefore, to calculate the latency of inhibition from the length of this interval and the latent period of the excitatory reflex.

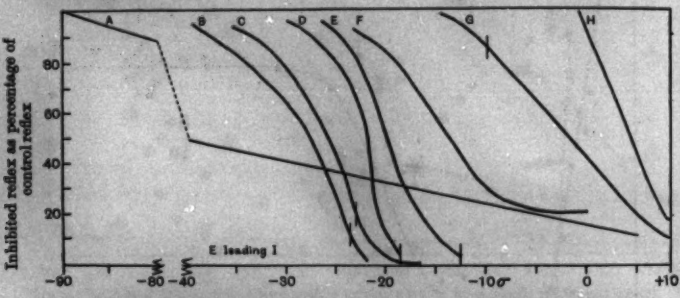

Fig. 5. Curves from different experiments, like Figs. 3 and 4, showing the relation of single-shock crossed extensor tension to *E–I* interval. The ordinates give the tension of inhibited reflexes on which each curve is based as a percentage of the average tension of the accompanying control reflexes. The vertical lines show the average latency of the uninhibited reflexes less peripherel conduction time. For significance see text.

In Fig. 5 vertical lines are drawn across the *E–I* curves at intervals from zero found by deducting from the latent period of the uninhibited reflexes the time required for peripheral conduction estimated at 6·5σ [Eccles and Sherrington, 1931*a*]. The distance of this line from the intersection of curve and base line is a measure of central inhibitory time[1]. In four of the curves the inhibition is almost complete at the interval indicated by the vertical line, and the base line would always be reached in a few stigmata, thus indicating the shortness of central inhibitory time. Since in this time are included both central conduction time and any delay for summation to reach an effective level, the latter must be very short.

The inhibitory stimulus was maximally effective in *G*, Fig. 5, only when it was given a lead of 10σ over the *E* shock, despite that in all other experiments *I* was effective when following the *E* stimulus. Even with regard to the short latent period of the *E* reflex,

[1] If *d* is the difference in central excitatory time (*e*) and central inhibitory time (*i*), i.e. $d=e-i$, then $d-e=i$; *d* and *e* can both be determined experimentally.

16·4 σ, it is surprising that the latency of inhibition seems actually longer. The central inhibitory time is calculated to be 19·9 σ. While the central discharge was undoubtedly highly repetitive, as judged by tension, duration and action current, an *I* stimulus affected these little provided the excitatory volley was given 10–20 σ lead. The central excitatory state underlying this reflex was presumably inhibitable only after a long period of inhibitory summation. Similarly, in the experiment for curve *F*, Fig. 5, in which inhibition was not maximal at any interval, the central inhibitory time was also much longer than in other experiments, being about 40 σ. The central time of an ipsilateral inhibition in quantities maximal for a given excitatory reflex may, therefore, be a few sigma or as long as 40 σ. Asynchronous inhibitory after-discharge would provide a basis for long central time, the figures given being the point in the inhibitory after-discharge at which sufficient impulses arrive at the anterior horn cell for complete inhibition.

The dependence of reflex tension on the length of the *E–I* interval, as shown in Figs. 3, 4, and 5, suggests a gradual encroachment of inhibition, when given earlier and earlier inception, on the period of central discharge, gradually eroding it away until complete extinction of the reflex is effected. If this is true, those reflexes which are initiated close before an inhibitory stimulus should suffer a shortening of duration as well as loss of tension as compared with an excitatory reflex which had been given an earlier start before the inhibitory stimulus. This curtailment of the contraction response does occur when the excitatory reflex is repetitive, as is shown by Figs. 6 and 7 *a*. The lessening of tension and of contraction time run roughly parallel up to a certain point, after which there is no further curtailment, though the tension continues to decrease. This point approximates to the contraction time and maximal tension of the motor twitch. In such experiments the temporal dispersion of the repetitive central discharge is in part responsible for the greater effectiveness of an early than a later inhibitory volley.

The lessening of contraction tension brought about by approaching the *I* to a preceding *E* stimulus is in many experiments not accompanied by significant alteration in duration of reflex response. This is illustrated by superimposed tracings in Fig. 7. When the reflex behaves to inhibition in this fashion a small decrease in the lead of *E* over *I* stimulus often suffices to change inhibition from a minimal to a maximal level, which contrasts with the long span of the *E–I* curve in Figs. 6 and 5 *A*. Further, the excitatory reflex is found to be of short duration and usually of small tension. The behaviour to inhibition confirms the evidence given above for the existence of single centrifugal volley crossed extensor reflexes or "reflex twitches." Though a single volley alone is produced, all or nearly all of the motoneurones may participate (Fig. 7).

There remains to ask the reason for the dependence of reflex tension on the length of the *E–I* interval when the reflex is of the "twitch" type.

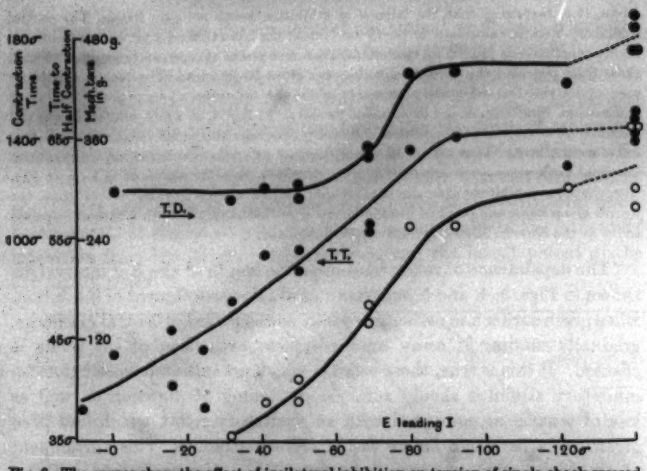

Fig. 6. The curves show the effect of ipsilateral inhibition on tension of single-shock crossed extensor reflex (dots), the time to half contraction (circles) and the time to point of maximal contraction (dotted circles). *T.T.* indicates tension, and *T.D.* the contraction time of the maximal motor nerve twitch of the recording muscle.

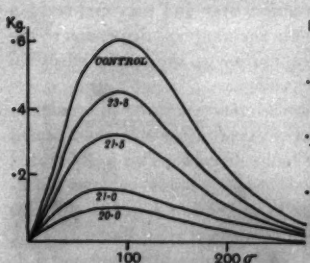

Fig. 7.

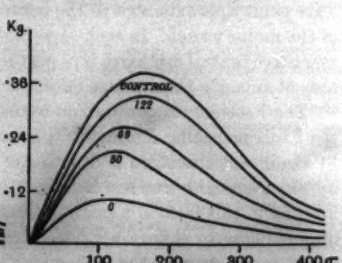

Fig. 7a.

Fig. 7. Superimposed tracings of single-shock crossed extensor reflexes inhibited to different extents by a single ipsilateral stimulus. The number attached to each curve is the interval in σ by which the *E* preceded the *I* stimulus. Shortness of contraction time (despite a strong tension development) is associated with absence of shortening by inhibition. *E* stimulus, 9 cm. and *I* stimulus 17 cm.

Fig. 7a. Same treatment of another experiment. *E* 17 cm. and *I* 11·5 cm. Maximum motor twitch was 200 g. Excess of contraction time over motor twitch is associated with a shortening of reflex discharge as the *E–I* interval is lessened.

In these reflexes temporal dispersion is confined to asynchronity of the single efferent volley. Asynchronity of the inhibitory volley in its arrival at the point of interaction with excitation probably combines with the temporal dispersion of the excitatory volley to make an early *I* stimulus more effective than a late one. When the excitatory reflex is of the "reflex-twitch" type, the slope of the *E–I* curve is probably largely fixed by the character of the inhibitory after-discharge. Thus, according to our observations, the *E–I* curve is determined by the character of both the excitatory and inhibitory volley.

The inhibition of single-shock crossed extensor reflexes differs from the contralateral inhibition of the ipsilateral flexor reflex as described by Eccles and Sherrington [1931 *c*] in these ways: complete inhibition of the single-shock crossed extensor reflex by a single inhibitory shock is more easily obtained; the span of the *E–I* curve is typically less when the excitatory reflex is of the "reflex twitch" type; the after-action of a single ipsilateral inhibitory stimulus is often briefer. These differences are consistent with the paths which inhibitory and excitatory volleys follow in the two kinds of experiment. After allowing for differences in the inhibition curves imposed by difference in the excitatory reflex, some of the same broad differences appear to exist between ipsilateral and contralateral inhibition that exist between ipsilateral and crossed excitation.

Conclusions.

1. Single afferent volleys are effective stimuli to contralateral extension in the chronic spinal cat, despite the "inertia" of conduction across the spinal cord and the depression of extensor reflexes in the spinal condition.

2. Single-shock crossed extensor reflexes are typically of short latency (20–35σ), though the latent period may be as short as 8·7σ or as long as 100σ.

3. One variety is of small tension (60–200 g.), of brief duration and myographic form not greatly different from the motor twitch, and is probably a single, asynchronous efferent-volley reflex. In a second variety, a repetitive central discharge is shown by a greatly prolonged mechanical and electrical response, and more rarely, by excess of reflex over maximal motor twitch tension.

4. The degree of inhibition of the reflex by an ipsilateral single shock depends on the interval separating the two stimuli. The level of inhibition increases rapidly as an ipsilateral stimulus is approached in time to a

preceding contralateral shock until an optimal interval is reached. Despite its ipsilateral path, inhibition wanes slowly when sampled at intervals by a following excitatory stimulus. Inhibition can, however, largely subside in a period as short as 25σ.

5. Inhibition can be used analytically to distinguish between reflexes of single asynchronous and repetitive central discharge.

In some experiments we had the pleasure of the collaboration of Prof. A. Forbes of the Harvard Medical School. We wish to express our gratitude to him and to Prof. Sherrington for much helpful advice. To the latter we owe the suggestion of making the spinal single-shock contralateral reflex of *soleus* in the spinal mammalian preparation the subject of investigation.

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RESPIRATION OF ISOLATED GILL TISSUE OF THE EEL.

By J. B. BATEMAN[1] and ANCEL KEYS[2].

(*From the Physiological Laboratory, Cambridge.*)

Introduction.

Various attempts have been made to show that osmotic regulation in marine and fresh-water animals is accompanied by increased respiratory exchange. The evidence is almost entirely presumptive; the respiration of a tissue is measured under conditions in which activity is assumed to occur without any proof of its occurrence; still further, from a direct correlation, the respiration of an entire animal is measured under two sets of conditions involving respectively quiescence and activity of the osmo-regulatory mechanism.

The results are open to some obvious objections. The animals may be more active, mechanically, under one set of conditions [Beadle, 1931; A. V. Hill, 1931, p. 69]—a criticism whose force is shown clearly by the experiments of Kreps [1929] on the respiration, in water of different salinities, of *Balanus crenatus*. Kreps found that cessation of motility was accompanied by a decrease of respiration to 1/3 or 1/4 of its normal value, while changes which could be attributed directly to changing salinity were of a much smaller order. Again, total respiration of an entire animal may be subject to complex regulatory influences which may mask the behaviour of any one organ. Schlieper has upheld the view that osmo-regulatory activity is accompanied by increased respiration of the animal and he has supported it by observations on the shore crab, *Carcinus maenas* [1929, 1930], and on *Gammarus locusta* [1931], and by the experiments of Tarussov [1927] on *Nereis diversicolor*[3]. In the first case the conditions for osmo-regulatory activity are well defined, but the question of muscular activity is a doubtful one; in the case of *Gammarus* there is no information concerning the behaviour of the body

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[3] Dr Schlieper informs us that he has recently obtained similar evidence in the case of some other forms, particularly *Eriocheir sinensis*.

fluids when the animal is in different environments, and therefore no experimental evidence in favour of Schlieper's interpretation. The evidence in the case of *Nereis diversicolor* derives some validity from Beadle's [1931] experiments, in which it was found that osmotic regulation occurs although it is absent in the closely related form, *Nereis cultrifera*. Other experiments of this kind are those of Beadle [1931] on *Gunda ulvæ*, the osmotic properties of which had been studied by Pantin [1931] and Weil and Pantin [1931], and the observations of Hayes [1930] on *Paramæcium*.

Where the osmo-regulatory process is probably more complex, as in the fishes, observations on the intact animals are still more difficult to interpret. Raffy and Fontaine [1930] reported that "civelles" adapted to fresh water showed a greater oxygen consumption than the same animals adapted to sea water, although a prolonged sojourn in fresh water presumably causes cessation of gill secretory activity. Keys [1931 *a*], on the other hand, found that the respiration of *Fundulus parvipinnis* is depressed in fresh water.

Schlieper [1929] measured the respiration of the isolated gill of the mussel, *Mytilus edulis*, and found that the gas exchange is greater in diluted media than in pure sea water. Here again, however, the observations are for the present of little significance, because there is no evidence that the gills of *Mytilus* play any rôle in osmotic regulation; it is not even known that osmotic regulation occurs in this animal.

The present experiments were undertaken in view of the possibility that the secretion of chloride, which is manifested by eel gills under certain conditions [Keys, 1931 *c*], might also occur in the extirpated gills under similar conditions. It was shown in Keys's experiments that the amount of secretory activity of the gills is determined by the internal concentration of chloride, so that no secretion is observed when the internal medium has a freezing-point depression of less than 0·50° C.; as the internal concentration is increased above this point the secretion is initiated and further small increases in the internal concentration provoke progressively larger increases in the secretion activity. These results have been confirmed by Krogh and Schlieper (unpublished experiments), who showed that the external concentration is not of itself a major factor in the phenomenon.

The conditions of internal concentration which maintain the gills active or passive as regards secretion can be brought about almost as readily with the gills extirpated as *in situ*. The perfusion technique worked out by Keys [1931 *b*] made it possible to fill the gills with fluid of any

desired concentration. When these gills are extirpated and suspended in an appropriate solution, diffusion and passive osmotic interchange between the internal and external solutions will tend to keep the internal concentration high or low, depending on the concentration of the external solution.

If the gills on one side of an eel are filled with Ringer's solution of $\Delta = 0{\cdot}70°$ C., extirpated and suspended in a balanced medium of high concentration, it is not too much to expect that they will be more active in secretion than the gills from the other side of the same animal, which are similarly perfused with a Ringer's solution of $\Delta = 0{\cdot}50°$ C. and suspended in a medium of low concentration. With preparations of this kind transferred to microrespirometers, it should be possible to obtain presumptive evidence for or against the utilization of oxygen in the branchial secretory process. It should be noted that the great exposure of surface in the gills makes them peculiarly suitable for respiration measurements.

Experimental.

The following account deals with the general procedure adopted in most of the experiments.

The eels (*Anguilla vulgaris*) used were kept in the laboratory in a tank of running tap water; some of these were transferred to sea water and allowed a few days for acclimatization before being used. The gills were perfused from the ventral aorta [Bateman and Keys, 1932], the cannula being connected by a two-way tap to two reservoirs containing respectively "concentrated" and "dilute" Ringer's solutions [prepared according to Keys, 1931 *b*] or different dilutions of sea water. The "concentrated" sea water had a freezing-point depression of 0·70° C. and the "concentrated" Ringer's solution a Δ of 0·72° C. The "dilute" sea water and the "dilute" Ringer's solution had freezing-point depressions of 0·50° C. and 0·55° C. respectively. In a few cases the animals were anæsthetized with amytal [Keys and Wells, 1930], but the amytal was washed out rapidly by the subsequent perfusion.

After perfusion with a given solution for some minutes, the gills on one side were exposed by cutting open the operculum and the afferent and efferent ends of the gill bars on this side were clamped off. At this time the reservoir tap was turned so as to perfuse the opposite side with the second solution. The first set of gills was excised by cutting away from the clamps and was suspended in the appropriate medium. After 10–15 minutes the second set of gills was excised similarly. The individual

gill bars were carefully separated and weighed in Ringer's solution after gentle blotting with clean filter paper. The fourth (posterior) bar on each side was discarded on account of its small size. It should be mentioned that in all these operations the gill filaments themselves were touched only in the process of removing excess liquid incidental to weighing, and, save at the extreme ends of the bar, the gill filaments suffered no manipulative damage whatever.

The gills perfused with the dilute and concentrated Ringer's solutions were now transferred to Barcroft manometers containing 3 c.c. 1/9 and 5/6 sea water respectively, and 0·5 c.c. 40 p.c. KOH. After a period of 20 minutes for temperature and gas equilibration, the taps were closed to the air and manometer readings taken at 15-minute intervals for several hours, bath temperature and barometric pressure being recorded. When the measurements were completed the gills were weighed after gentle blotting, re-weighed after being heavily blotted to remove all adherent moisture, and finally the gill filaments were dissected away from the cartilaginous bar and the weight of the latter obtained. This dissection could be made quite cleanly and the proportion of relatively inert cartilage and non-filament tissue in the gill was obtained sufficiently accurately for an adequate correction to be made in computing the respiration of the gill-filament tissue proper. The respiration of the "cartilage" alone was determined in several experiments, and a mean value used in the application of this correction. The constants for the Barcroft manometers were corrected in each experiment for the different volumes of liquid and tissue used.

Proportion of gill respiration due to "cartilage."

The figures for "cartilage" respiration are given in Table I. The values for each hour of experiment are the means of the four 15-minute measurements. The mean values, 54·3 c.mm. O_2 per g. per hour for the first hour and 49·4 for the second, were used in all calculations. The

Table I. Respiration of gill cartilage at 15° C.

Exp.	Mean respiration in c.mm. O_2 at N.T.P. per g. per hour: First hour	Second hour
C 1	65·8	52·6
C 2	74·4	86·5
C 3	45·5	30·3
C 4	45·7	40·1
C 5	39·9	37·3
Means	54·3	49·4

rather wide variation in the individual values is probably due to incomplete removal of filament tissue, making the lower figures more probably correct. But the corrections involved are small enough for the mean value of all experiments to be used without serious error.

The proportion of the measured weight of gill bars due to "cartilage" was found usually in each individual case, but, as this determination was sometimes neglected, a mean value had to be obtained from all the figures available. These seem to be worth presenting, since they illustrate the cleanness with which the dissection of cartilage could be done (Table II). The mean value, 48 p.c., was used in calculation.

TABLE II. Proportion of cartilage in gill bar.

Series No.	No. of determinations	Mean weight of cartilage as p.c. of total blotted dry weight
P 1	6	48
P 2	6	47
P 3	6	49
P 4	6	42
P 5	2	44
P 6	2	51
P 7	4	43
P 8	4	50
P 9	2	54

The experimental procedure and method of calculating results may now be made clearer by a detailed typical protocol. The remaining data will be presented more briefly by graphs and summarizing tables.

Protocol of Exp. 2. 25. iii. 1932.

Eel from fresh water after 2 days in laboratory. Operation begun 12.17 p.m., perfusion begun 12.20 p.m. Operation complete 12.22 p.m. Internal perfusion media: 1/3 sea water from start till 12.53 p.m., 1/4 sea water till 1.5 p.m. External medium: tap water. Gill from right side dissected out at 12.52 p.m. and transferred temporarily to 1/3 sea water. Left side out at 1.5 p.m. and transferred to 1/4 sea water. Placed in respiration chambers at 1.15 p.m., each apparatus containing 3·0 c.c. external medium and 0·5 c.c. 20 p.c. KOH. The manometer readings were continued for 4·5 hours, then the tissue was weighed. The details of external and internal media, weights of tissues, and manometers are given in Table III, while Table IV shows the detailed figures for one manometer, together with the essential stages in the calculation.

The constant k for manometer 75, determined at 15·5° C. and 763 mm. and containing 0·1 c.c. liquid, was 3·08, or 2·90 at N.T.P. For this experiment, with cups containing 3·5 c.c. liquid + 0·177 g. tissue at 14·3° and 760 mm., k becomes 2·43. Then the rate of O_2 uptake is kx (see Table IV). This is due partly to respiration of gill filaments and partly to cartilage. Correction for the latter is applied by subtracting from kx the product (weight of cartilage) × (mean respiration rate of cartilage) = B, or 0·085 × 51·8 = 4·6 (see Tables I and III). The true respiration of gill tissue is then this difference divided by the weight of gill tissue (M). In Table V the results of the calculation are given for all six manometers.

TABLE III. Weights of tissue, etc., in EXP. 2.

1. Apparatus No.	75	76	78	114	116′	116
2. Internal medium	Dilute Ringer's solution			Conc. Ringer's solution		
3. External medium	1/9 sea water			5/6 sea water		
4. Weight of gills at end of exp.: g.	0·177	0·182	0·121	0·158	0·161	0·134
5. Weights of gill bars calc. as 0·48 × (4)	0·085	0·087	0·058	0·076	0·077	0·064
6. Weights of gill filament tissue (M)	0·092	0·095	0·063	0·082	0·084	0·070

TABLE IV. Calculation of respiration rates in EXP. 2.

Manometer 75. $k = 2·43$. Weight of gill tissue 0·092 g.
Cartilage correction, $B = 4·6$ throughout.

Time (min.)	Rate of movement of manometer liquid: $= x$ mm./hour	kx c.mm. O_2 per g. per hour	$kx - B$	$\frac{kx - B}{M}$
0– 15	16·8	40·8	36·2	394
15– 30	14·0	34·0	29·4	320
30– 45	11·2	27·2	22·6	246
45– 62	11·3	27·5	22·9	249
62– 90	11·4	27·7	23·1	251
90–105	9·6	23·3	18·7	203
105–120	11·6	28·2	23·6	256
120–135	10·8	26·3	21·7	236
135–150	10·0	24·3	19·7	214
150–165	12·0	29·2	24·6	267
165–180	10·0	24·3	19·7	214
180–215	9·9	24·3	19·7	214
215–245	10·0	24·3	19·7	214
245–275	9·2	23·3	18·7	203

TABLE V. Final results for EXP. 2.

Respiration rates for gill tissue in c.mm. O_2 per g. per hour at N.T.P. Temperature = 14·3° C.

	Internal medium "dilute" Ringer's solution. External medium 1/9 s.w.			Internal medium "concentrated" Ringer's solution. External medium 5/6 s.w.		
Manometer No.	75	76	78	114	116′	116
Time (min.)						
0– 15	394	217	351	458	392	456
15– 30	320	285	276	473	408	433
30– 45	246	252	320	416	424	261
45– 62	249	228	198	358	286	224
62– 90	251	194	220	365	321	252
90–105	203	252	232	372	240	188
105–120	256	194	246	401	348	247
120–135	236	194	246	358	339	321
135–150	214	182	203	386	332	203
150–165	267	205	173	372	348	203
165–180	214	194	246	328	332	247
180–215	214	235	198	379	336	228
215–245	214	171	195	328	324	166
245–275	203	171	218	328	282	240

s.w. = sea water.

Results.

The figures obtained in the detailed protocol, which are plotted in Fig. 1, are typical. In all other experiments of the same kind the respiration was definitely greater when the liquid bathing the gills was sea water or 5/6 sea water than when it was tap water or 1/9 sea water. Individual manometers very occasionally, as in the experiment quoted, gave irregular results, which could as a rule be traced to an imperfectly greased tap. In Fig. 2 the results of a similar experiment are plotted, the ordinate here being total O_2 uptake per g. gill tissue instead of the rate which is plotted in Fig. 1. This shows the main effect rather more strikingly.

Table VI. Respiration of isolated gills in 1/9 s.w. and in 5/6 s.w.

Experimental series	Internal media	Mean respiration rate in c.mm. O_2 per g. per hour: 1/9 s.w.	Mean of series	5/6 s.w.	Mean of series	Increment, rate in 5/6 s.w. minus rate in 1/9 s.w.	Increment as p.c. of values in 1/9 s.w.
2	"Dilute" *R* in 1/9 s.w.	274·0	256·3	406·1	348·7	92·4	36
	"Conc." *R* in 5/6 s.w.	231·7		345·6			
		263·3		294·4			
3	1/4 s.w. in 1/9 s.w.	68·7	77·3	120·0	116·2	38·9	50
	1/3 s.w. in 5/6 s.w.	86·0		112·5			
4	Same as series 3	215·5	211·3	227·0	301·5	90·2	43
		211·5		344·5			
		207·0		333·0			
10	Same as series 2	286·0	288·1	308·7	323·1	35·0	12
		289·0		337·5			
		289·2					
11	Same as series 2, plus glucose and urea	269·5	208·6	387·7	344·4	135·8	66
		204·5		367·5			
		151·9		278·0			
16	Same as series 2	220·6	220·6	386·6	386·6	148·0	67
						Mean of all	46 p.c.

Each value given is the mean of from 6 to 12 determinations; a total of 234 determinations is summarized in this table. *R* = Ringer's solution.

s.w. = sea water.

The remaining experiments are summarized in Table VI, in which the mean rates of respiration are given for each manometer used, over a period of 2 hours. In column 7 the differences between the mean rates in the two media are given for each experiment, this difference being expressed in column 8 as a percentage of the "normal" respiration in 1/9 sea water. It is clear that there is a consistent, though rather variable, positive difference, amounting on the average to about 46 p.c. of the respiration in 1/9 sea water. The variability is to be expected in a

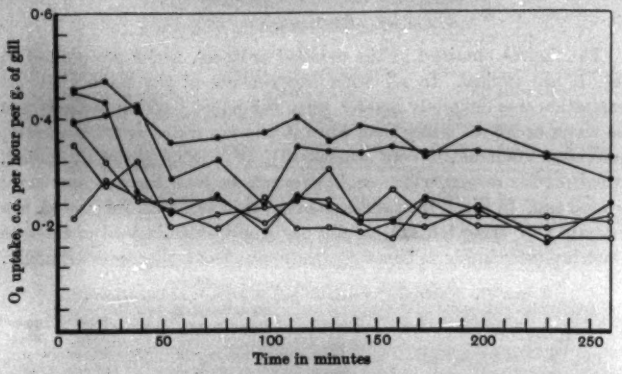

Fig. 1. Effect of salt concentration on the respiration of extirpated gill tissue. Rates of respiration of six gills from a single eel. Solid circles, gills perfused with Ringer's solution $\Delta = 0{\cdot}70°$ and suspended in 5/6 sea water. Open circles, perfused with Ringer's solution $\Delta = 0{\cdot}50°$ and suspended in 1/9 sea water. Temp. 14·3°.

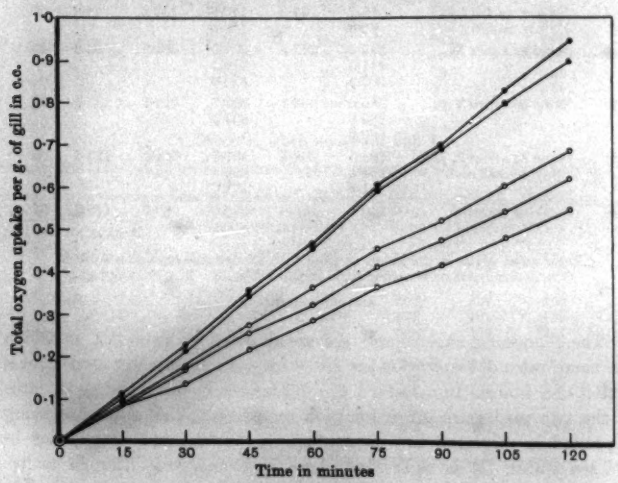

Fig. 2. Effect of salt concentration on the respiration of extirpated gill tissue. Total respiration of five gills from a single eel. Solid and open circles as in Fig. 1. Temp. 14·6°.

tissue subjected to such drastic manipulation before the performance of the experiment. There was generally a tendency for the respiration to decrease somewhat during the course of the experiment; this may be seen in Fig. 1, which is typical of the behaviour observed. The data are not, however, sufficiently extensive to indicate any tendency of the increment due to salt concentration to decrease more rapidly than the total respiration.

The existence of the effect under slightly more physiological conditions was confirmed in a series of experiments on the blood-filled gills. The procedure was as follows: the eel was pithed and the gills exposed with care to avoid serious bleeding. The afferent and efferent ends of the gills were clamped with hæmostats, as in the previous case, and the gills excised with the clamps still in position. Then the individual gill bars were ligatured at both ends, the clamps removed, and the gill bars cut apart. The respiration experiments were carried out as in the other experiments and the results are summarized in Table VII. In Fig. 3

TABLE VII. Respiration of blood-filled gills in dilute and concentrated media.

Experimental series	Previous habitat of eel	External solution	Resp. rate	Mean rate	External solution	Resp. rate	Mean rate	Increment, conc. minus dilute rate	Increment, as p.c. of dilute rate
5	s.w.	f.w.	146·0 127·2	136·6	s.w.	290·0 218·5 472·5	327·2	190·6	140
7	f.w.	,,	213·5	213·5	,,	368·5	368·5	155·0	71
8	,,	,,	158·2	158·2	,,	223·5	223·5	65·3	41
9	,,	,,	97·0	97·0	,,	228·7	228·7	131·7	135
12	,,	1/9 s.w.	177·5 128·9	153·2	5/6 s.w.	206·2 244·7	225·4	72·2	47
15	s.w.	,,	190·0 187·7	188·8	,,	289·0 241·5	265·2	76·4	40
								Mean of all	79 p.c.

s.w. = sea water; f.w. = fresh water.

the data of experiments 7, 8 and 9 are plotted. In each of these three experiments a small eel was used, and the whole gill from one side put into one respiration chamber. It appears that, although in a single experiment the respiration was more constant under these conditions than in the perfused gills, the actual O_2 consumption was somewhat smaller and the increase of respiration in sea water even more marked, having a mean value 79 p.c. more than that of the respiration rate in fresh water. For gills from eels acclimatized to sea water the mean is 76 p.c. and for those from fresh water it is 82 p.c.; considering the wide variation in the quantities observed this agreement may be fortuitous.

The most rigid proof of the reality of the phenomenon under discussion would be to show the effects of salt concentration on the respiration of the same portion of tissue. Such an experiment would put a severe strain on the capacity of the extirpated tissue to adjust to the changing concentration of its environment, and it could hardly be expected that

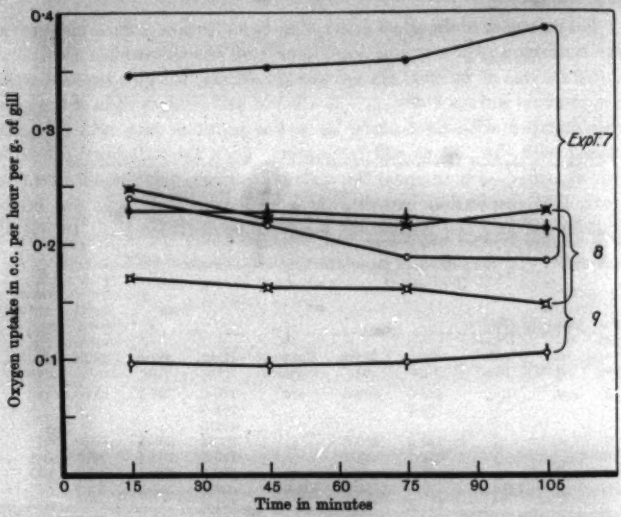

Fig. 3. Effect of salt concentration on the respiration of extirpated blood-filled gills. Three experiments, eels from fresh water. Solid circles, gills suspended in sea water. Open circles, gills suspended in tap water. Temp. 14·0°.

the secretory mechanism would respond perfectly under these circumstances. Experiments, however, showed that under these conditions there is an effect of salt concentration on the oxygen consumption which, although small, is in agreement with the earlier experiments; *i.e.* the oxygen consumption tends to be higher in the more concentrated environment. Table VIII summarizes the results of these experiments in which the respiration of individual gills was measured first in an environment of one concentration and then in an environment of a very different concentration.

TABLE VIII. Effect of change of salt concentration on the respiration of individual gills.

Group A, internal concentration initially $\Delta = 0{\cdot}70°$ C.

Gill No.	First observations, relative resp. rate in environment $\Delta = 1{\cdot}7°$ C.	Second observations, relative resp. rate in environment $\Delta = 0{\cdot}3°$ C.
1	100	82·0
2	100	86·5
3	100	104·0
4	100	81·0
5	100	71·0
6	100	85·5
7	100	92·0
Means	100	86·0

Group B, internal concentration initially $\Delta = 0{\cdot}48°$ C.

Gill No.	Second observations, relative resp. rate in environment $\Delta = 1{\cdot}7°$ C.	First observations, relative resp. rate in environment $\Delta = 0{\cdot}3°$ C.
8	100	94·5
9	100	97·5
10	100	78·0
11	100	91·0
12	100	83·0
13	100	84·5
14	100	86·5
Means	100	87·9

DISCUSSION.

The increase in respiration with increase in the salt content of the environment has been observed regularly under different experimental conditions, and its reality seems certain. Its interpretation in terms of the performance of secretory work by the gills is simple and attractive, but it is necessary to consider the possibility that some other effect of changing salinity may be involved.

The effects observed in the various experiments cannot be ascribed to any difference in the *p*H of the solutions. The initial *p*H of the internal solutions was always between 7·5 and 7·7, and the *p*H of the external solutions was between 7·8 and 8·0; the shift to greater alkalinity due to abstraction of CO_2 in the respiration chambers was practically the same in the various solutions. As to the normality of the tissue respiration observed, we may mention that the effect of cyanide on the gill respiration was quite normal.

In some cases it appears that tissue respiration, even where no secretory activity is known to occur, may be a function of the salt concentration of the environment, but there is no general ground upon which pure osmotic effects on respiration can be predicted. The effect of

hypertonic NaCl solutions in increasing the respiration of fertilized sea-urchin eggs [Warburg, 1909; Meyerhof, 1911], for example, was supposed by Warburg to be a direct effect of NaCl on the respiration, while Schlieper [1931] attributes it to an attempt to prevent, by an active osmotic regulation, the entry of the poisonous NaCl. With plant cells increase of internal osmotic concentration produces no predictable general effect [Smith, 1915–16; Palladin and Sheloumova, 1918; Inman, 1921]. Hayes [1930] found that the respiration of *Paramœcium* is increased both in diluted and in concentrated media. Clearly, in these cases Schlieper's explanation of the Warburg sea-urchin eggs experiment cannot be applied. In the present instance, calculation shows it to be quite possible that the decreased respiration in dilute sea water may be to some extent an injury effect due to salt loss to the surroundings; if we reason thus we must conclude that the tissue in 5/6 sea water suffers to an even greater extent from dehydration. The dilute sea water (Δ = about 0·23° C.) is much nearer the normal internal osmotic pressure of the gills (Δ = about 0·6–0·7° C.) than the concentrated sea water (Δ = about 1·65° C.). Moreover, the observed respiration of gill tissue immersed in 1/3 sea water is rather less than in 5/6 sea water, as is shown in Table IX.

TABLE IX. Respiration of gills in 1/3 s.w. (Δ = 0·67° C.).

Respiration rates in c.mm. O_2 per g. per hour. Exp. series				Mean rate	Mean respiration rate of gills in 5/6 s.w.
A	B	C	D		
427	324	208	237	292	302
407	304	226	264		
411	263	212	246		

s.w. = sea water.

In the attempt to discover whether there is a general effect of salt concentration on the respiration of eel tissues, we have studied the respiration of the thin membranous fins, which, like the gills, are well suited for respiration measurements and are subjected to great variation in the external concentration in the normal life cycle of the animal. The technique was similar to that used in the gill experiments; the fins were extirpated and the respiration of the fin from one side of the eel was measured in 1/9 sea water, while the respiration of the corresponding fin from the other side was measured in 5/6 sea water. In each experiment eight determinations were made over a period of 2 hours. The results of the six experiments are given in Table X; there is no trace of a consistent effect of the salt concentration.

TABLE X. Respiration of eel fins in 1/9 and in 5/6 s.w.

	Relative respiratory rates per g. per hour	
Exp. No.	In 1/9 s.w.	In 5/6 s.w.
F 1	100	93·5
F 2	100	102·0
F 3	100	121·0
F 4	100	99·0
F 5	100	85·5
F 6	100	113·0
Means	100	102·0

Eight determinations were made in each experiment.

s.w. = sea water.

The data presented in this paper have clearly the defect of some of the earlier experiments on the respiration of secretory tissue; they do not show, unequivocally, that the performance of work is causally associated with increased oxygen consumption. One can only point out that the tissue has a proven secretory capacity, and that the respiration does increase under conditions which are known to be associated with the initiation and maintenance of the secretion. Assuming, for the moment, that the secretion hypothesis is the correct one, we can make some more detailed observations on the experiments reported.

If the osmotic pressure of the internal medium really provides the stimulus for the secretion, then we must be able to show that the necessary change in the internal environment in the case of the blood-filled gills can occur early in the experiment in which the external medium is 5/6 sea water. We may use data for the permeability of the perfused gills when fresh water is the external medium [Keys, 1931 *b*]. These data indicate a water entry of about 5 c.c. per kg. eel per hour. In eels of the size used in the present experiments this means an entry of roughly 0·15 c.c. of water per hour per gill. The gill volume may be put at 0·5 c.c. (probably less) of which 20 p.c. is osmotically inert substance. It is clear that the normal permeability will effect in 1 hour a change of at least 35 p.c. in the internal concentration. Where the concentration gradient is greater, as in the case of gills suspended in 5/6 sea water, the effect of passive diffusion will be proportionally greater. The permeability of the extirpated gills, moreover, may be taken to be considerably greater than that of the gills *in situ*, in the same way that any tissue subjected to manipulation shows an increased permeability. It is obvious that sufficient exchange will take place in the early stages of the experiment to stimulate or stop the secretory activity as the case may be. The

results, then, are consistent with the opinion that the internal osmotic pressure is the stimulus for the secretion. This conclusion has been reached also from the recent experiments by Krogh and Schlieper [1932].

The respiration increment due to, or associated with, higher salt concentration is an excess of oxidation over the "resting" value in the lower salt concentration, and the excess energy yielded in this way may be calculated and compared with the medium energy cost of the gill secretion observed in the perfusion experiments. If it could be assumed that the salt concentration stimulus evoked the same degree of secretion activity in the extirpated gills as in the perfused gills, such a calculation would provide an answer to the question as to the efficiency of the secretory mechanism. Actually, of course, it is highly probable that the secretory activity in the extirpated gills is smaller, perhaps much smaller, than in the perfused gills, and the result of our calculation will be of interest only as a limiting value.

The mean value for the "secretion" increment in all experiments is 102·6 c.mm. O_2 per g. per hour. Assuming that carbohydrate is burnt, the combustion of this amount of oxygen would yield 0·51 cal. The secretion observed in the perfusion experiments involves a performance of thermodynamic work amounting to 0·1–0·3 cal. [Bateman and Keys, 1932], with the mean value not far from 0·15 cal. The first approximation to the maximum efficiency is therefore 29 p.c.

Organs capable of specialized activity frequently have a high resting metabolism. It is of interest, therefore, that the respiration of eel gill tissue in a dilute medium—about 210 c.mm. O_2 per g. per hour at 15° C.—is proportionally considerably greater than that of the mammalian lung. Lovatt Evans [1912] gives 500[1] c.mm. O_2 per g. per hour at 36° C. for the respiration of the lung tissue in the heart-lung preparation, and this works out to be about 1·5 times the rate for the body as a whole. The respiration rate of the "resting" gills is about seven times that of the resting intact eel, as measured by Krogh [1904], while the respiratory rate of the gill in the concentrated medium is about ten times that for the entire animal. Moreover, if allowance is made for the temperature difference by the application of the usual temperature coefficient—Q_{10} about 2·0—the respiratory rates of "resting" and "active" gills become respectively 100 p.c. and 190 p.c. greater than the rate of the mammalian lung tissue. For purposes of comparison, we have

[1] The value obtained from Evans's paper by Bayliss [1924, p. 612] appears to be incorrect.

assembled data on the respiratory rates of various mammalian and eel tissues in Table XI.

TABLE XI. Respiration of various tissues.
Respiration rates in c.mm. O_2 per g. per hour of wet tissue.

Tissue	Mean respiratory rate	Observer
Mammalian kidney	1600–3600	Neumann, 1912
Mammalian kidney	Approx. 1680	Barcroft and Brodie, 1905
Mammalian suprarenal gland	2750	Neumann, 1912
Mammalian submaxillary gland	1920	Verzár, 1912
Mammalian submaxillary gland	1620	Barcroft and Piper, 1912
Mammalian lung	500	Evans, 1912
Mouse and rat diaphragm	1000–2000	Meyerhof, 1930
Frog, small muscles at 14·8° C.	59	Fenn, 1927
Eel, pectoral fin at 15° C.	78	Original
dorsal fin (strips) at 15·1° C.	61	,,
central fin (strips) at 15·1° C.	67	,,
mid gut (strips) at 15·3° C.	63	,,
blood-filled gills in 1/9 s.w. at 14·8° C.	167	,,
blood-filled gills in 5/6 s.w. at 14·8° C.	273	,,
gills after perfusion, in 1/9 s.w. at 15·0° C.	210	,,
same calculated for 37° C.*	1000	,,
gills after perfusion, in 5/6 s.w. at 15·0° C.	302	,,
same calculated for 37° C.*	1430	,,

* Assuming Q_{10} is 2·0.

SUMMARY.

1. The respiration of excised eel gills, filled with Ringer's solution and blood, has been measured in dilute and concentrated external environments.

2. The percentage of gill filament tissue in the gills has been determined and the respiration of the bars minus the filaments measured.

3. The mean oxygen uptake of the gills filled with Ringer's solution, corrected for the respiration of the relatively inert cartilaginous bars, was 210 c.mm. O_2 per g. per hour with 1/9 sea water as the external medium and 300 c.mm. with 5/6 sea water. In all experiments the respiration was consistently greater in the more concentrated medium.

4. With the blood-filled gills an even greater difference was found between the respiration in 1/9 and 5/6 sea water, the respiration in the latter averaging 80 p.c. greater than in the former.

5. Possible causes of this concentration effect are discussed and reasons are given for the belief that it is related to secretory activity in the gill. The possible efficiency of this activity is discussed.

6. The fins of the eel do not show any effect of osmotic concentration of the environment on the respiratory rate.

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THE MUCUS FACTOR IN THE AUTOMATIC REGULATION OF THE ACIDITY OF THE GASTRIC CONTENTS.

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The automatic regulation of the gastric acidity is the result of the interaction of several factors. These are (1) intragastric, which are brought into play by the stomach itself; and (2) extragastric, which originate from without, that is to say from the duodenum and œsophagus. Study of a mass of clinical cases demonstrates quite clearly that each of these factors is of varying importance in different pathological conditions. It is, therefore, essential for the elucidation of such conditions that, in the first instance, the part played by each of these factors in the normal individual should be precisely defined. This research is concerned with the intragastric factors alone and more particularly with gastric mucus. This factor has hitherto been largely disregarded in favour of a hypothetical diluting solution, which has recently been regarded by some as a secretion [Maclean and Griffiths, 1928], in fact gastric juice in which the HCl has been replaced by NaCl. As a preliminary study of the intragastric factors, we, therefore, investigated the acidity of the gastric juice during a secretory wave and were able to prove that the original view of Pavlov was correct [Bolton and Goodhart, 1931; Pavlov, 1910]. We found that the HCl content of the gastric juice as secreted by the cell varies a little with the rate of secretion, but that the main cause of the moderate variations in acidity which occur during secretion is neutralization and dilution by mucus. Neither by any automatic conversion of HCl into NaCl by the secreting cell, nor by admixture with mucus is the unaided stomach able to reduce materially the acidity of the gastric juice during its secretion. We showed, however, that when secretion has stopped the reaction of the residual fluid in the stomach gradually becomes alkaline owing to dilution and neutralization with mucus. Since mucus is thus, in some degree, a factor of importance and

the precise part played by it is unknown, the next step in our study is clearly to determine the degree to which mucus is capable of neutralizing solutions of HCl of different amounts and strengths.

METHOD.

Cats were used, and in all the experiments the stomach was isolated from the rest of the alimentary canal by ligature of the cardia and pylorus. For all details of preparation of the cat the reader is referred to the above-mentioned communication [Bolton and Goodhart, 1931]. It is merely necessary to mention here that the HCl solution was introduced into the isolated stomach through the side tube of a wide cannula, the mouth of which was closed with a cork. The residual fluid, which would not flow out of the stomach after emptying it, was removed by a small glass syringe and piece of tubing introduced into the mouth of the cannula, through which easy access to all parts of the stomach was assured. The fluid was measured on introduction and after extraction. It is not possible to remove the whole of the fluid with certainty, owing to small amounts being held up by the folds of mucous membrane. Sometimes the measurements are the same, but usually a fraction of a c.c. less than the volume introduced is extracted in spite of the addition of a little mucus. If any considerable amount of fluid is added by the stomach, however, it can be recognized quite easily.

A fresh cat was used for each experiment, so that the compensatory changes incident to a permanent operation and liable to lead to erroneous results were avoided, and also each animal was in a thoroughly healthy condition. A large number of animals, to allow for normal variations, was used, and the stomach of each was examined after the experiment.

I. NEUTRALIZATION EFFECTS AS REGARDS VOLUME AND CONCENTRATION OF HCl SOLUTION.

In studying the effects of mucus, one must be quite certain that the gastric glands are at rest and remain so during the experiment. Although the cat has been isolated and has received no food for 24 hours, the mucous membrane of the stomach is acid in reaction in a considerable number of animals, and there is no certainty of its becoming alkaline within a reasonable time. Furthermore, we have found, as will appear later, that the secretion of gastric juice may continue or be excited by the acid solution introduced. In order to produce a pure mucous effect we, therefore, administered atropine gr. 1/10 subcutaneously after the

animal had been prepared. The secretion of gastric juice is thus promptly stopped, that of mucus continuing, and the mucous membrane gradually becomes alkaline first to dimethylamidoazobenzol and later to litmus. On the average it took about 25 min. before alkalinity to litmus was developed; variations about this average occurred due to different rates of mucus secretion, which varies from cat to cat. It is obvious that the longer the acid solution remains in the stomach the greater will be the reduction of its acidity; and also that the reduction to alkalinity of even small quantities would occupy a time outside the limits of our experiments. For comparison of the effects upon the different solutions the standard time of a quarter of an hour was selected, during which the fluid was allowed to remain in the stomach. It was then removed for analysis and replaced by a similar amount of fresh solution of the original strength; a curve of reduction of acidity was thus produced. The concentration of the solutions was approximately that of gastric juice, and also a descending series below this level.

GROUP 1. ATROPINE EXPERIMENTS. (TWENTY-ONE EXPERIMENTS.)

(1) *Acidity of* HCl *solution introduced of the same concentration as gastric juice.*

Acidity 0·140–0·144 *N*. *Total* Cl 0·14 *N* (0·5 *p.c.*) (Fig. 1).

When the amount introduced is 10 c.c. a slight fall in acidity occurs of about 0·01 *N*; the total Cl also falls by about 0·004 *N* (0·014 p.c.). Larger amounts show less effect still. In view of this fact four experiments were performed using amounts of 10, 5 and 2 c.c. introduced every quarter of an hour.

Amount 10 *c.c.* The quarter-hourly curve remains horizontal as regards both acidity and total Cl. The same amounts as those introduced were extracted or a fraction of a c.c. less.

Amount 5 *c.c.* The same result was obtained on two occasions. The acid curve was reduced by 0·05 *N* at the beginning and by 0·03 *N* at the end, and therefore showed a slight gradual rise. The total Cl curve was horizontal and only reduced by about 0·01 *N* (0·036 p.c.); the first two or three specimens being slightly more affected than the others. The same amount or a fraction of a c.c. more than introduced was withdrawn on each occasion.

Amount 2 *c.c.* The acid curve was reduced by 0·06 *N* at the beginning and 0·05 *N* at the end, and the total Cl curve by about 0·014 *N* (0·043 p.c.).

The reduction in acidity is, therefore, only a marked feature when

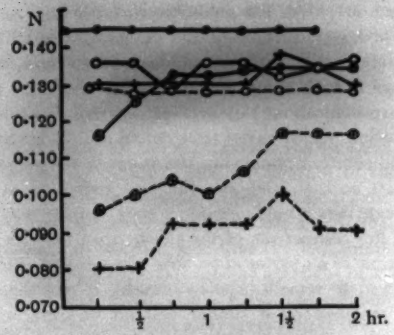

Fig. 1. Cat under atropine; 0·14 *N* HCl introduced every ½ hour.

Total Cl and total acid introduced ●——●——●

Gastric contents, total Cl: 10 c.c. introduced every ½ hour ○——○——○
5 c.c. „ „ ⊕——⊕——⊕
2 c.c. „ „ +——+——+

total acid: 10 c.c. „ „ ○ - - - ○ - - - ○
5 c.c. „ „ ⊕ - - - ⊕ - - - ⊕
2 c.c. „ „ + - - - + - - - +

In all experiments under atropine gr. ¼ was injected subcutaneously and HCl introduced as soon as the mucous membrane had become alkaline to litmus.

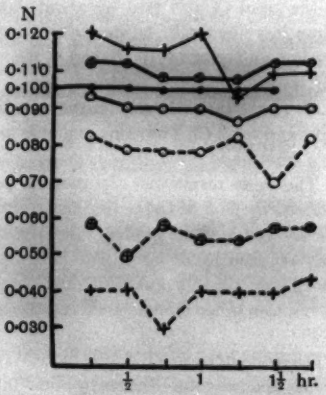

Fig. 2. Cat under atropine. 0·106 *N* HCl introduced every ½ hour. Symbols as in Fig. 1.

amounts below 10 c.c. are introduced and is a little less after repeated introduction. This latter event is due to continual washing of the mucous membrane with acid and is without doubt the same phenomenon as is observed in the acidity of the secretory curve, which is lower at the beginning than the end of secretion. With weak solutions of acid this effect is not seen. On the other hand the total Cl curve falls but little, and practically to the same degree in each case. This is owing to the high Cl content of mucus, which is only slightly less than that of gastric juice, and which, therefore, acts only feebly as a diluting fluid.

(2) *Acidity of* HCl *solution introduced of a concentration below that of the gastric juice.*

Acidity 0·1–0·11 *N*. *Total* Cl 0·1–0·11 *N* (0·36–0·39 *p.c.*) (Fig. 2).

Amount introduced 10 *c.c.* The acidity curve was reduced by about 0·010 *N*, but the total Cl curve was unaffected.

Amount 5 *c.c.* Both curves were horizontal, the acidity being reduced by 0·03 *N* and the total Cl raised by from 0·004 to 0·008 *N* (0·014–0·028 p.c.).

Amount 2 *c.c.* The reduction of acidity was about 0·06 *N* and the rise of total Cl 0·004–0·008 *N* (0·014–0·028 p.c.).

The acidity showed a greater reduction with the smaller amounts than the larger, and to about the same degree as when the concentration of the HCl was 0·140 *N*. On the other hand the total Cl was raised a little in the case of the smaller amounts, since the diluent, mucus, has a greater concentration of total Cl than the fluid introduced.

Acidity 0·068–0·072 *N*. *Total* Cl 0·06–0·07 *N* (0·24–0·25 *p.c.*) (Fig. 3).

Amount introduced 10 *c.c.* The acid curve was again reduced slightly by about 0·004–0·01 *N* and the total Cl raised by about 0·004–0·008 *N* (0·014–0·028 p.c.).

Amount 5 *c.c.* The reduction of acidity was by about 0·02 *N* and the rise in total Cl by 0·02–0·03 *N* (0·07–0·1 p.c.).

Amount 2 *c.c.* The result was almost the same as in the preceding experiment. Reduction of acidity by 0·03 *N* and rise in the total Cl by 0·02 *N* (0·07 p.c.).

It is to be noted that the reduction of acidity is less than in the case of the same amounts of the stronger solutions, but the rise of total Cl is more, owing to the greater difference between the concentration of Cl in the introduced fluid and in mucus.

Acidity 0·036–0·044 *N*. *Total* Cl 0·036–0·044 *N* (0·129–0·15 *p.c.*) (Fig. 4).

Amount introduced 10 *c.c.* The acidity was reduced by 0·015 *N* in one case and by 0·004 *N* only in the other. On the other hand the total Cl percentage went up by 0·036 in the former and 0·1 in the latter.

Amount 5 *c.c.* The fall in acidity was 0·02 *N* in one case, but only 0·004–0·008 *N* in the other. The total Cl percentage rose by 0·1 in the former and 0·07 in the other.

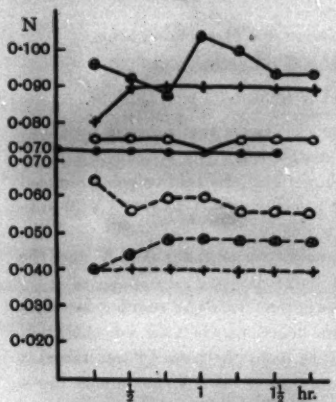

Fig. 3. Cat under atropine. 0·072 *N* HCl introduced every ½ hour. Symbols as in Fig. 1.

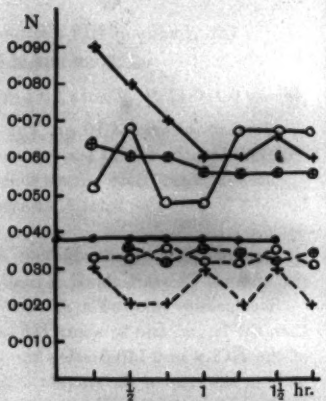

Fig. 4. Cat under atropine. 0·038 *N* HCl introduced every ½ hour. Symbols as in Fig. 1.

Amount 2 *c.c.* The reduction in acidity was only 0·006–0·016 *N* and the rise in total Cl percentage by 0·19 at the beginning, and by 0·08 at the end.

Generally speaking, in the case of strong solutions up to the strength of gastric juice, both acidity and total Cl fall, the former more than the latter; and the smaller the volume of solution the greater the fall in acidity, but the total Cl fall remains about the same in all cases. In the case of weak solutions the acidity falls, but less than with strong solutions, and the total Cl rises. The weaker the solution the less the fall in acidity and the greater the rise in total Cl. The difference between the effects upon acidity in the case of large and small amounts of weak solutions

is less marked than in the case of the strong solutions: but the total Cl is always higher with small amounts of weak solutions. However small the volume of stomach contents the reaction is invariably acid, and after complete emptying the mucous membrane becomes alkaline in about half an hour. These effects are all referable to two facts: (1) The total Cl content of mucus which is the diluent is very little less in most cases than that of gastric juice, so that below a certain level of total Cl content of introduced solution, say 0·36 p.c., the rise in total Cl is greater the weaker the solution and the smaller the amount present. (2) The neutralizing power of mucus depends upon the relative amount present. The smaller the amount of solution the greater the relative amount of mucus. The stronger the solution the greater the flow of mucus, and the greater its relative amount.

(3) *Reproduction of secretory curve.*

This demonstration that the gastric mucus lowers the acidity of definite amounts of definite strengths of HCl solution to approximately definite levels, and that so long as the acid solution is renewed the level remains constant, implies that a gradually rising and falling curve of acidity can be produced by appropriately varying the amounts of the acid solution. In order to test this implication a series of four experiments was undertaken, atropine gr. 1/10 being administered to produce alkalinity of the mucous membrane.

The concentration of the HCl solution was 0·14–0·144 *N*, the strength of gastric juice. The amounts introduced each quarter of an hour were 2, 5, 4, 3, 2 c.c. in two cases, 2, 6, 4, 3, 2 c.c., and 3, 6, 5, 4, 3 c.c. in the other two cases (Fig. 5). The acidity fell from its initial height in two cases to 0·08 *N* and in the others to 0·06 and 0·07 *N* respectively during the first quarter-hour period. At the end of the experiment the acidity of the final amounts extracted in three cases stood from 0·01 to 0·024 *N* higher than at the beginning: in the final case it stood at 0·12 *N*, 0·05 *N* higher than at the beginning and remained at this level after three further introductions of the same amount (2 c.c.). A rising and a falling acidity curve was thus produced in three cases and a climbing curve in the other, the final acidity being always higher than at the beginning of the curve. The total Cl curve was about 0·01 *N* (0·036 p.c.) or less below the level of that introduced and was a horizontal line in three cases: in the other case it fell finally to 0·024 *N* (0·084 p.c.) below the initial level. This fall was accounted for by the low Cl content of the mucus of this animal: the mucous membrane after emptying the stomach became

alkaline in half an hour, and the mucus extracted contained only 0·36 p.c. inorganic chloride, rather lower than usual.

These four experiments reproduced precisely the same type of curve as is obtained during the secretion of gastric juice. The effect of the mucus was, however, more marked, so that the acidity curve ran at a lower level than is usual with the gastric secretory curve. The explanation is obviously that the gastric juice, secreted as a continuous process, has to pass through the overlying mucus which it saturates before appearing free in the stomach, so that, as Pavlov puts it, the mucous membrane becomes well washed. This is a more thorough process than the intermittent introduction of acid solution into the stomach lined with mucus, so that the neutralization effect is less marked in the case of the secreted acid.

Group 2. Control experiments without atropine. (Six experiments.)

In all the above experiments under atropine the only secretion by the stomach was mucus to which the neutralization effect was due; and, furthermore, after the stomach had become emptied, this was proved to be the case by collecting the subsequent secretion. It is still possible that the stomach might secrete a neutral diluting fluid unless stopped by the atropine. A series of similar experiments was, therefore, performed without using atropine, the HCl solution introduced being of the same strength as gastric juice, about 0·14 *N*. The stomach was very gently mopped free of residual fluid with absorbent cotton wool before introducing the solution. If the mucous membrane was acid in reaction we found that it might remain so for a couple of hours, so we made no attempt to wait till it became alkaline, but introduced the solution irrespective of the initial reaction of the mucous membrane. We shall return to this point later when describing another series of experiments. There were three experiments in which the same amounts—20, 10 or 5 c.c.—were repeatedly introduced and three in which increasing and diminishing amounts were used—2, 5, 4, 3, 2 c.c., 3, 6, 5, 4, 3 c.c. (Fig. 5), and 2, 4, 3, 2 c.c. The amounts extracted on each occasion were similar to those in the experiments under atropine.

Amount 20 *c.c.* There was a very slight reduction of acidity of 0·004 *N*, an insignificant amount (Fig. 6).

Amount 10 *c.c.* There was a great deal of mucus in this case and the reduction of acidity at the beginning was 0·028 *N* and at the end of the curve 0·008 *N* (Fig. 6).

Amount 5 *c.c.* The acidity of the first specimen was reduced by 0·036 *N* and at the last by 0·02 *N* (Fig. 6).

Each of the second three experiments reproduced the type of curve which we have called the secretory type, the final acidity being less reduced for the same amount of solution than the initial was. For example the cases showed:

Amounts c.c.	Acidity of first specimen *N*	Final acidity *N*
2	0·1	0·11
3	0·09	0·11
2	0·05	0·08

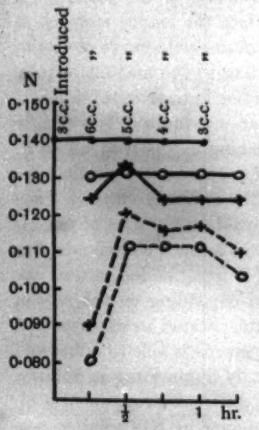

Fig. 5.

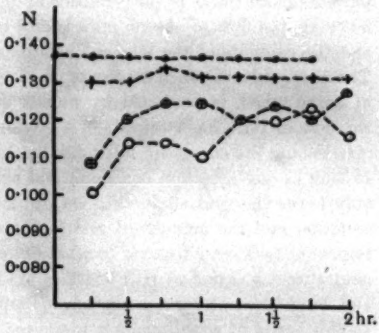

Fig. 6.

Fig. 5. Reproduction of secretory curve by introduction of increasing and diminishing amounts of 0·14 *N* HCl.

Cat under atropine, gastric contents: total Cl ○——○——○
total acid ○ - - - ○ - - - ○
Cat without atropine, gastric contents: total Cl +——+——+
total acid + - - - + - - - +
Total Cl and total acid introduced ●——●——●

Fig. 6. Control cats without atropine. 0·137 *N* HCl introduced every ½ hour.

Total acid introduced ● - - - ● - - - ●
Gastric contents: 20 c.c. introduced, total acid + - - - + - - - +
10 c.c. „ „ ⊕ - - - ⊕ - - - ⊕
5 c.c. „ „ ○ - - - ○ - - - ○

These results are the same as those in the atropinized animal and were very uniform considering the fact that slight acid secretion was occurring at the time of the experiment in each case. It is reasonable to conclude that the neutralization effect in these cases was due to mucus and that no neutral diluting secretion was concerned.

II. NEUTRALIZATION EFFECTS IN RELATION TO THE EMPTYING OF THE STOMACH.

We have hitherto considered the effects of mucus upon HCl solution of different amounts and strengths which were constantly renewed. The results of these observations are directly applicable to the pathological condition of hypersecretion of gastric juice into the empty stomach at different rates. If the rate of secretion is maintained the curve of acidity is a horizontal line at different levels according to the amount of juice put out in unit time. In the condition of digestion in the normal stomach, however, the flow of gastric juice comes to an end more or less rapidly and the contents of the stomach are diminishing as the food leaves it. The next problem which confronts us, therefore, is to ascertain the type of curve which is produced by mucus as the stomach empties when the flow of juice has stopped; it is obvious that as the flow of juice is diminishing the curve will fall similarly, but at a much slower rate, for, so long as any secretion continues and acid is being added, mucus can only lower the acid curve to a certain level depending on the rate of secretion and the amount of gastric contents. Stated in experimental terms our task is to discover to what extent mucus is able to dilute and neutralize a solution of HCl which is gradually diminishing in volume, and what types of curve are produced with different strengths of acid. In this series of experiments a measured amount of HCl solution was introduced into the stomach, and each quarter of an hour the whole of the solution was removed and measured. A certain volume was retained for analysis and the remainder returned into the stomach. The concentration of acid used was that of the gastric juice, also weaker and stronger solutions. Two groups of experiments were performed: (1) under atropine, to obtain a pure mucus effect; (2) a control series, without atropine.

Group 1. Under atropine gr. 1/10. (Eight experiments.)

Concentration of HCl = 0·140–0·144 *N*. *Total* Cl 0·14–0·144 *N* (0·50–0·51 *p.c.*) (Fig. 7).

When the mucous membrane had become alkaline 50 c.c. of the solution were introduced, and a quarter of an hour later the stomach

was emptied; 40 c.c. of the fluid thus removed were immediately returned to the stomach, and each quarter hour subsequently the process was

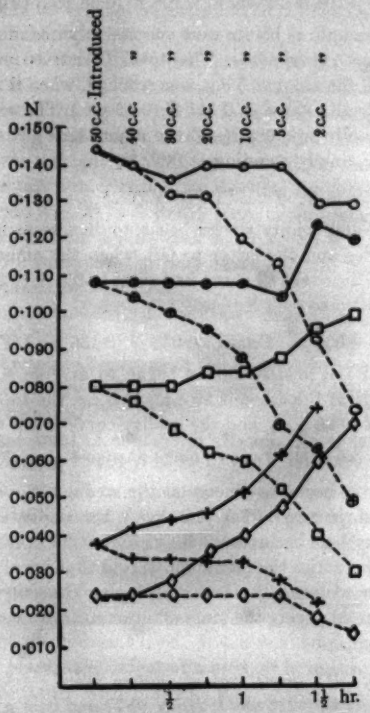

Fig. 7. Five cats under atropine. 50 c.c. HCl, 0·144 *N*, 0·108, 0·080, 0·040, 0·024 *N* introduced; stomach emptied at ½ hour and 40 c.c. of gastric contents put back at once, and so on. Gastric contents: total chloride, continuous line; total acid, interrupted line.

repeated, 30, 20, 10, 5 and 2 c.c. being returned at each successive period. The total Cl curve was constant till the amount diminished to 10 or 5 c.c., when it fell by about 0·036 p.c. The acid curve began to fall slightly at the 20 or 10 c.c. amounts: at the amount 5 c.c. it had reached 0·097–

0·1 *N* and at 2 c.c. 0·075 *N*. These levels correspond quite well with those of the earlier experiments.

Concentration = 0·108 *N*. *Total* Cl 0·108 *N* (0·38 *p.c.*) (Fig. 7).

The same amounts as before were successively introduced in this and all the following experiments. The total Cl curve remained at the original level till the amount 5 c.c. was reached, when it rose to 0·123 *N* (0·44 p.c.) and finally stood at 0·117 *N* (0·42 p.c.). The acidity curve fell slightly and steadily to 0·088 *N* till the amount 20 c.c. was reached; it then fell rapidly, finally standing at 0·05 *N*.

Concentration = 0·08 *N*. *Total* Cl 0·08 *N* (0·28 *p.c.*) (Fig. 7).

The total Cl and acidity curves began to diverge as in the last case, the former to rise and the latter to fall, when the amount 30 c.c. was reached; and at 2 c.c. the Cl curve had risen to 0·1 *N* (0·36 p.c.) and the acidity curve fallen to 0·03 *N*.

Concentration = 0·038 *N*. *Total* Cl 0·038 *N* (0·136 *p.c.*) (Fig. 7).

The total Cl and acidity curves began to diverge slightly at once, and more rapidly at the amount 20 c.c.; at 5 c.c. the total Cl curve had risen to 0·073 *N* (0·26 p.c.), and the acidity curve fallen to 0·022 *N*.

Concentration = 0·024 *N*. *Total* Cl 0·024 *N* (0·084 *p.c.*) (Fig. 7).

The two curves began to diverge at the amount 30 c.c., and at 2 c.c. the Cl curve had risen to 0·07 *N* (0·25 p.c.); the acidity curve remained at its original level till the amount 5 c.c., and at 2 c.c. it fell to 0·014 *N*. Here again in this series the fact is illustrated that lower concentrations of HCl are reduced in acidity less than higher concentrations. In this experiment after emptying the stomach, the mucous membrane became alkaline in 20 min.

Concentration of HCl *above that of gastric juice. Acidity* 0·216 *and* 0·276 *N*. *Total* Cl 0·216 *and* 0·276 *N* (0·77 *and* 0·99 *p.c.*).

In each case the amounts introduced were 50, 30, 10, and 5 c.c.

The results of these two experiments differ from the foregoing in the following particulars:

Concentration 0·276 *N*:

(1) From 1 to 2 c.c. were extracted in excess of that introduced on each occasion.

(2) The diluting fluid was an inflammatory effusion containing albumin and blood.

(3) From the beginning, when the amount was 50 c.c., there was a sharp fall of both total Cl and acidity, the latter outpacing the former. At the end of the experiment the total Cl stood at 0·16 *N* (0·561 p.c.), having fallen from its original height of 0·276 *N* (0·993 p.c.); the acidity had meanwhile fallen to 0·1 *N*.

Concentration 0·216 *N*. This case showed less marked features than the former:

(1) An excess of fluid was removed in the last two specimens only.

(2) The diluting fluid was an inflammatory effusion containing blood.

(3) There was a steady fall of both curves from the beginning, but only as steep as the former case after reaching the amount 10 c.c.; the final total Cl level was 0·172 *N* (0·619 p.c.), having fallen from 0·216 *N* (0·77 p.c.); and the final acidity was 0·116 *N*. So that the acidity fell to practically the same level in both cases and the final total Cl percentage only differed by 0·05 p.c.

The following deductions may be drawn from a consideration of this series of cases:

(1) When the total Cl content of the solution is greater than that of mucus the total Cl curve falls, the rapidity of fall depending upon the strength of the solution.

(2) When the total Cl content of the solution is less than that of mucus the curve rises.

(3) In each case when alkalinity is reached after emptying the stomach, the total Cl level stands at that of mucus, or of the inflammatory effusion in the case of solutions much stronger than gastric juice.

(4) This fall or rise to a common level of Cl is not due to absorption or output of Cl in order to regulate the Cl content of the solution in the stomach to a definite constant level. It is due to admixture with mucus, and the final Cl percentage is that of mucus, or in the case of very strong solutions to admixture with inflammatory effusion, the Cl content of which is about the same as mucus.

(5) The rapid fall with very strong solutions, and the less rapid fall with solutions of the strength of gastric juice and of strengths above it to a certain height, are due to the quantity of diluting and neutralizing fluid. This quantity depends on the concentration of HCl in the solution and reaches its maximum in the cases showing inflammatory effusion.

(6) A whole series of diminishing degrees of both rapidity of fall, and final level reached, is seen in the acidity curves of the different strengths of acid:

(*a*) In the case of weak solutions up to the strength of gastric juice, a fall of acidity only occurs to any degree, when small amounts of solution are introduced. Above the strength of gastric juice the fall affects larger amounts of fluid, till, when the stage of inflammatory effusion is reached, a rapid fall occurs at the 50 c.c. amount.

(*b*) The rate of fall gradually diminishes and the curve becomes less steep from the higher to the lower strengths.

(*c*) The degree of fall, and the final level reached, is less and less marked as the strength of the HCl diminishes.

There is thus a whole series of irritation effects produced according to the strength of the HCl; for it is only reasonable to conclude, that the increasing fall of acidity with increasing strengths of HCl, when mucus secretion alone is excited, is due to increasing irritation, since the series passes up gradually into the cases of inflammatory effusion obviously due to local irritation of the HCl.

GROUP 2. CONTROL CASES WITHOUT ATROPINE. (THIRTY EXPERIMENTS.)

In this second group of experiments the solutions were introduced into the isolated normal stomach after removing any fluid which might have been present. Atropine was not administered, so the results observed were comparable to the neutralization effects which may reasonably be expected to occur during the emptying of the normal stomach, as the result of intragastric factors alone. This series of experiments is thus of great importance. There were three series as in the atropine cases. The amounts introduced were 50, 40, 30, 20, 10, 5 and 2 c.c.

(A) Concentration of acid similar to that of normal gastric juice, 0·135–0·152 *N*.

(B) Concentration of acid below this level, 0·02–0·12 *N*.

(C) Concentration of acid above this level, 0·188–0·292 *N*.

(A) *Concentration of acid similar to that of normal gastric juice*, 0·135–0·152 *N*. *Total* Cl 0·135–0·150 *N* (0·48–0·54 *p.c.*) (Figs. 8 and 9).

Precisely the same results were obtained as in the atropine cases. The total Cl curve remained almost unaffected till the amount 10 c.c. was reached, when a fall began, the height standing at 0·125 *N* (0·45 p.c.) when the amount 2 c.c. was reached, a fall of about 0·023 *N* (0·08 p.c.). The acid curve was very little affected till the 10 c.c. amount, when it began to fall rapidly, reaching the level of about 0·09 *N* at the 5 c.c. amount, and 0·07 *N* at the 2 c.c. amount. In two cases the emptying

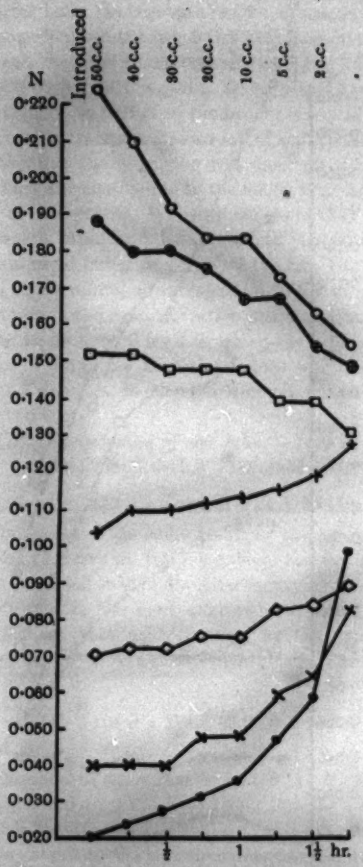

Fig. 8. Seven cats. Control experiments of Fig. 7 without atropine. Gastric contents, curves of total Cl.

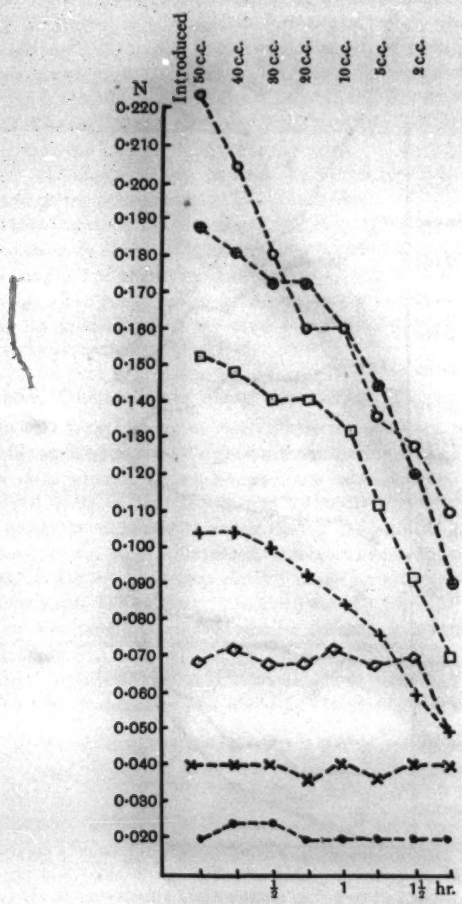

Fig. 9. Same as Fig. 8. Curves of total acid.

time was lengthened by 1 hour, by withholding smaller amounts than usual each quarter hour. In one case the 2 c.c. amount was reached at the end of 2¾ hours, and in the other the 20 c.c. amount was reached in 2¼ hours. In each the total Cl curve was very little affected, falling by 0·06 in one and 0·08 p.c. in the other. In each case there was a steady uniform fall of acidity, to 0·04 *N* in the former case and to 0·09 *N* in the latter. In each case there was a greater fall of acidity than occurred with similar amounts when the emptying was more rapid, but the fall was more gradual. This was clearly due to the longer time which was allowed for the action of the mucus. We may conclude that the quicker the emptying the more rapid and less complete the fall; the slower the emptying the less rapid but more complete the fall. The rate of emptying of the stomach is, therefore, an intragastric factor which influences the acidity of the contents and in the direction just mentioned. Furthermore, there appears to be no special level to which the acidity is regulated by the mechanisms of emptying time and mucus secretion, the final level is fortuitous, and depends upon relative amounts of acid solution and mucus.

(B) *Concentration of acid below* 0·02–0·12 *N*.
Total Cl 0·02–0·12 *N* (0·07–0·43 *p.c.*).

Concentration of acid = 0·12 *N*. *Total* Cl 0·12 *N* (0·43 *p.c.*).

This experiment gave the same result as the atropine experiments, in that the total Cl curve at the 2 c.c. level rose to 0·13 *N* (0·46 p.c.) and the acidity curve fell to 0·05 *N*. After emptying the stomach, 0·5 c.c. mucus was collected in the next quarter-hour period and showed an acidity of 0·02 *N*, so that neutralization by the mucus was not yet complete. This, as we have already mentioned, takes on the average half an hour.

Concentration = 0·096–0·104 *N*. *Total* Cl 0·09–0·104 *N* (0·32–0·374 *p.c.*) (Figs. 8 and 9).

Typically the total Cl curve rose to 0·11–0·117 *N* (0·396–0·42 p.c.) at the 5 c.c. amount and to 0·125 *N* (0·45 p.c.) at the 2 c.c. amount. The acidity curve fell to 0·06–0·084 *N* at 5 c.c., and to 0·05 *N* at 2 c.c. This is a quite similar result to the corresponding case under atropine.

Concentration = 0·068–0·084 *N*. *Total* Cl 0·068–0·084 *N* (0·24–0·29 *p.c.*) (Figs. 8 and 9).

These curves showed a less marked effect than the corresponding case under atropine and did not diverge until the amount 10 c.c. was

reached. The total Cl rose to 0·084–0·1 *N* (0·29–0·36 p.c.) at the 5 c.c. amount. The acidity at this time in one case did not fall at all; in another it rose to 0·076 *N* from a level of 0·068 *N*; and in the others fell slightly to about 0·06 *N*.

Concentration = 0·02–0·04 *N*. *Total* Cl 0·02–0·04 *N* (0·07–0·14 *p.c.*) (Figs. 8 and 9).

These curves similarly differed from the atropine cases in showing no fall in acidity; for example, it showed no fall at all in three, and in one it rose slightly. The total Cl rose in all by from 0·02 to 0·04 *N* (0·07–0·14 p.c.) at the 5 c.c. amount. The stomach was emptied in one case, and after ¾ hour 1 c.c. of mucus was extracted showing an acidity of 0·07 *N* and total Cl of 0·14 *N* (0·5 p.c.).

These irregularities in acidity in the last two series can only be due to one thing, namely the secretion of acid gastric juice.

We now bring together the cases showing evidence of this secretion.

(1) The acid curve rises instead of falling. Two experiments:

(*a*) The acidity of the 50 c.c. amount introduced was 0·068 *N* and the total Cl 0·068 *N* (0·23 p.c.). When the 5 c.c. amount was reached the acidity had risen to 0·076 *N* and the total Cl to 0·08 *N* (0·288 p.c.).

(*b*) The acidity of the 50 c.c. of introduced fluid was 0·035 *N* and the total Cl 0·035 *N* (0·12 p.c.). When the 5 c.c. amount was reached the acidity stood at 0·045 *N* and the total Cl at 0·055 *N* (0·198 p.c.).

(2) In one case the secreted fluid which was collected ¾ hour after emptying the stomach had a higher acidity than the introduced fluid. The acidity of the original fluid introduced was 0·04 *N* and total Cl 0·04 *N* (0·14 p.c.). When the 2 c.c. amount was reached the acidity still remained at 0·04 *N*, and the total Cl had risen to 0·083 *N* (0·29 p.c.). The mucus, which was collected after emptying the stomach, had an acidity of 0·07 *N* and a total Cl of 0·14 *N* (0·5 p.c.).

(3) In three cases the acidity remained at the same level throughout, that is to say 0·04, 0·038, and 0·02 *N*. No fall in acidity occurred in any of the series of cases of low acidity, and although we cannot prove that this is due to secreted acid it is most likely to be the cause, for in no case did such an event occur in the atropine cases. When solutions of acidity stronger than that of the last series of cases is employed, it is not possible to say whether or not there is any secretion of gastric juice. It is only when the acidity is at this low level that the small amounts of gastric juice secreted could add to the acidity appreciably, otherwise it could do no more than retard the fall of the acid curve: or when the mucoid

fluid extracted after the stomach is emptied has an acidity greater than the original acidity can it be said that there would be proof that acid was being secreted.

It has already been mentioned that there is no evidence of any mechanism regulating the acidity of the gastric contents to any special level. It might be argued that the rise of acidity when weak solutions are introduced, and the fall of acidity with strong solutions, means that the stomach is trying to adjust the acidity of its contents to some intermediate level. This cannot be so, for most of the weaker solutions show no rise, the curve being a horizontal line; and when a rise occurs it is to no definite level. Also the fall of acidity in the case of the stronger solutions is finally to alkalinity, and does not stop at any definite level of acidity. In all cases when the stomach is emptied the secretion obtained is usually alkaline mucus, but may be mucus with some secreted gastric juice. Similar remarks apply to the total Cl curve which is not adjusted to any definite level, although it falls slightly in the case of strong solutions and rises with weak solutions. The final level when alkalinity is reached is somewhat variable, depending on the concentration of Cl in the mucus.

(C) *Concentration of acid* 0·188–0·292 *N*.
Total Cl 0·188–0·292 *N* (0·66–1·04 *p.c.*).

Concentration of HCl (0·188 and 0·196 *N*). *Total* Cl 0·188 and 0·196 *N* (0·66 and 0·7 *p.c.*) (Figs. 8 and 9).

Both these cases showed a fall in the acidity and total Cl curves from the beginning, but the fall in total Cl was quite gradual from start to finish, the final reading being, at the amount 5 c.c., 0·15 and 0·153 *N* (0·54 and 0·55 p.c.) in the two cases, so that they fell by 0·034 and 0·04 *N* (0·12 and 0·14 p.c.) respectively. The acid curve fell more rapidly after the amount 10 c.c., and at the 5 c.c. amount stood at 0·120 and 0·125 *N* in the two cases, so that they fell by 0·068 and 0·071 *N* respectively. This result is so nearly like that of Group 1 that it must be due to the same cause, and a comparison of the two curves leaves no doubt in the mind that the difference in the two cases is due to greater mucus secretion in the case of the stronger solution, owing to greater irritation. We could not collect enough mucus afterwards to prove that it did not contain albumin, but it will be seen later than albumin does not usually occur with these strengths of HCl.

Concentration = 0·224, 0·276, 0·292 *N*. *Total* Cl 0·224, 0·276, 0·292 *N* (0·8, 0·99, 1·047 *p.c.*) (Figs. 8 and 9).

In each case both the curves fell steeply from the beginning, more especially that of the highest strength. The two cases 0·276 and 0·292 *N* gave the same results, in that a greater amount was extracted than was introduced, more particularly at the amounts 40, 30 and 20 c.c. when it was from 3 to 6 c.c. in excess. This was proved to be due to admixture with an inflammatory exudate containing about the same amount of total chloride as mucus, that is 0·125 *N* (0·45 p.c.). At the 5 c.c. amount the total Cl had dropped to 0·142 and 0·148 *N* (0·51 and 0·53 p.c.): the total acidity at the same time stood at 0·05 and 0·06 *N* respectively. These results are practically the same as those of the atropine experiments. In the case 0·224 *N* no more was extracted than was introduced on each occasion, and we could not obtain sufficient mucus for examination, so are unable to say whether it contained albumin. When the 5 c.c. amount was reached the total Cl stood at 0·16 *N* (0·56 p.c.) and the acidity at 0·11 *N*; not such a great fall in acidity as with the stronger solutions.

Reviewing these three groups of experiments, it is plain that they show the same transition of results as occurs in the atropine cases. The fall in acidity is proportionate to the strength of the solution: the same fall in total Cl occurs in the strong solutions and the same rise in the weak solutions: the neutralizing and diluting fluid is the same in each case, having a quantity and quality proportionate to the strength of the acid; mucus with strengths some degree above that of gastric juice, and inflammatory effusion with higher strengths still. The only difference is that gastric juice is liable to be secreted, as shown in the case of the weaker strengths of acid; whether this is a continuation of the slow secretion going on in the fasting stomach of the cat, and unaffected by the HCl solutions introduced, or whether it is newly excited by the solution, perhaps by the water it contains, we are unable to say.

III. MUCUS.

This section deals with the direct evidence we have obtained that mucus alone is the diluting and neutralizing fluid of these HCl solutions, and that when a certain concentration of acid is reached an inflammatory effusion is added to the mucus. This inflammatory fluid is of no moment from the physiological point of view, but for our purpose it is of importance, because it is clearly due to irritation by the HCl; and since it

gradually becomes mixed with and succeeds the mucus secretion, and the effects of the one gradually pass into the other, it is only reasonable to suppose that the similar graduated series of effects due to mucus alone, and varying in accordance with the strength of the acid, are dependent upon the varying amounts of mucus secreted in response to the irritation of the HCl. Furthermore, these experiments show how comparatively easy it is to produce such an inflammatory effusion, which is of great importance in pathology.

When an irritant fluid is applied to the mucous membrane of the stomach a flow of mucus is excited, and continues for a time after the fluid is removed, so that it may be collected. This is the method we have employed for the collection of mucus in this series of experiments. It is, however, only on occasion that sufficient may be collected for analysis after removal of these solutions of HCl. The mucus is thick and sticky and considerable quantities adhere to the mucous membrane and are entangled in its folds, but it is only when it collects in a pool in the cavity of the stomach that it can be extracted. The stomach was emptied as far as possible and then any remaining fluid was gently removed with absorbent cotton wool. The mucous membrane was tested from time to time as to its reaction; it became alkaline first to dimethylamidoazobenzol, and about half an hour later to litmus. At this stage the mucus was allowed to collect until sufficient could be extracted. We succeeded in obtaining it from twenty cases, but in the rest, although the mucous membrane became alkaline, no definite quantity of fluid could be extracted. We have already referred to one case in which the mucous membrane did not become alkaline but secreted gastric juice, and have given further evidence of the secretion of this fluid, so need not refer to it again. Of the twenty experiments ten were performed under atropine and ten without atropine. There was no difference in the two series; in all cases mucus only was obtained or an inflammatory exudate containing albumin, and there was never any sign of the secretion of a neutral diluting fluid. There were eight cases of inflammatory exudate and twelve of mucus secretion. The amount of inflammatory exudate put out in from ½ to 1 hour was 4–6 c.c. It contained albumin and often blood pigment, and the total Cl content was the same as that of mucus. Mucus is not secreted so abundantly; in five cases from 1 to 5 c.c. were collected in about an hour, and in seven no more than 0·5–0·6 c.c. The physical characters are the same as those of mucus obtained by other forms of irritation. The viscidity varies a little; sometimes the mucus runs well, at other times not. It is slightly opaque and contains a pro-

teose, and the alkalinity was 0·04 *N* (0·14 p.c.). The chloride content more particularly concerns us, and the analysis of the five above-mentioned cases is as follows:

Amount of mucus collected c.c.	Total Cl concentration	Concentration of HCl
1	0·1 *N* (0·36 p.c.)	0·04 *N*
1·5	0·12 *N* (0·43 p.c.)	0·044 *N*
1	0·14 *N* (0·50 p.c.)	0·07 *N*
1·5	0·12 *N* (0·43 p.c.)	0·104 *N*
1	0·131 *N* (0·47 p.c.)	0·148 *N*

The analysis of the seven cases in which smaller amounts were collected, and which are not, therefore, so reliable, is as follows:

Amount of mucus collected c.c.	Total Cl concentration	Concentration of HCl
0·6	0·12 *N* (0·43 p.c.)	0·02 *N*
0·7	0·14 *N* (0·50 p.c.)	0·038 *N*
0·3	0·128 *N* (0·46 p.c.)	0·140 *N*
0·4	0·11 *N* (0·39 p.c.)	0·144 *N*
0·5	0·1 *N* (0·36 p.c.)	0·144 *N*
0·5	0·12 *N* (0·43 p.c.)	0·145 *N*
0·6	0·14 *N* (0·50 p.c.)	0·180 *N*

The chloride content of gastric mucus excited by various strengths of HCl, therefore, varies from 0·36 to 0·5 p.c., and is independent of the strength of the HCl solution. This Cl content of mucus explains the rise or fall of total Cl in the introduced HCl solutions, which we have already described. We found the same percentage of total Cl in the mucus secreted in response to pilocarpine stimulation. The concentration of HCl giving rise to inflammatory exudation is about 0·19–0·2 *N* and all strengths above this level. In such cases hæmorrhages and erosions to various extents were seen in the mucous membrane of the stomach, or recognized microscopically.

IV. SUMMARY AND CONCLUSIONS.

1. The only means possessed by the normal stomach, whereby it is able to reduce the acidity of its contents, is by the secretion of mucus.

2. The action of mucus is influenced by two additional intragastric factors; the rate of emptying of the stomach and most important of all the cessation of secretion of gastric juice.

3. The more rapidly the stomach empties the more rapidly does the curve of acidity fall, notwithstanding that a shorter time is allowed for the action of the mucus than in the slowly emptying stomach. The

amount of gastric contents is thus of greater importance than the time allowed for the action of mucus in this neutralization process.

4. As regards amount to be neutralized, there is very little reduction of acidity till the amount is reduced below 10 c.c. in the cat.

5. As regards time for the action of mucus, a greater final reduction of acidity occurs for any definite amount of fluid in the slowly emptying than in the rapidly emptying stomach. However, a very considerable time is required for the reduction to alkalinity of quite a small amount of fluid, for, after complete emptying of the stomach, about half an hour is required to render the mucous membrane alkaline.

6. The importance of the cessation of gastric secretion for the neutralization process lies in the fact, that, so long as secretion continues, mucus produces no reduction of acidity unless very small amounts are being secreted. In this case the acidity falls according to the amount present and remains constantly at this level.

7. The acidity of a weak solution of HCl is reduced by a smaller amount than that of a strong solution. This is due to the irritant action of HCl, which produces a larger secretion of mucus according to its concentration.

8. The eventual end result of this neutralization process is always a fall to alkalinity, facilitated by emptying of the stomach, and rendered possible by the cessation of secretion of gastric juice. There is no mechanism regulating the acidity to any definite level, either by stoppage in the fall of acidity or output of acid to raise it.

9. The total Cl percentage is not regulated to any definite level. The final Cl percentage is not reached till the contents are alkaline, and it then stands at the variable level of that of mucus. There is no evidence whatever of the secretion of neutral chloride to raise the level of Cl percentage in the gastric contents, or of a diluting fluid other than mucus to lower it to any definite concentration. Unless the amount of gastric contents is small enough to be affected by mucus there is no attempt whatever to raise or lower the total Cl content or the acidity.

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THE HISTAMINE-HISTAMINASE SYSTEM IN THE ISOLATED PERFUSED KIDNEY-LUNG PREPARATION.

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In an earlier paper [MacGregor and Peat, 1931] we showed that stimulation of the sympathetic or parasympathetic nerve supply to the lungs in the intact cat produced no detectable changes in the histamine content of the lung, using the method of assay based upon the depression of a cat's blood-pressure. In a further endeavour to arrive at some conclusions regarding the significance of the presence in the body, in relatively large amounts, of such a potentially active substance as histamine, the investigation to be described was undertaken.

The discovery by Best [1929] of an enzyme system which specifically destroyed histamine and which was found to be present in many organs suggested to us the study of the relationship between this enzyme system and the naturally occurring histamine of the lung. Lung tissue itself contains this "histaminase," since Best [1929] found that on incubation of minced lung tissue in saline the naturally occurring histamine of the lung was rendered physiologically inert. Best and McHenry [1930] showed later that the kidney (at least in the dog) provided a much richer source of the enzyme. As a first step in our investigation we set out to find if a similar inactivation of the histamine occurred when the isolated but intact lung of the dog was perfused at 37° C. with defibrinated blood.

I. Isolated perfused lung experiments.

The lungs were perfused with a Bayliss [Bayliss and Müller, 1928] pump under positive pressure ventilation; they lay on a gauze filter in a funnel which allowed the blood to drain freely from the open pulmonary veins. The pulmonary artery pressure was kept constant at 30 cm. of blood. The whole apparatus was enclosed in a thermostat. The dogs were anæsthetized with chloroform and ether with the exception of a few cases where nembutal was used. The lungs were washed through with defibrinated blood before being removed from the chest. In these and in

all the experiments to be described later the lung of one side only was perfused, that of the opposite side for the purpose of control being extracted with alcohol immediately on removal from the thorax.

The lung was weighed before and after the perfusion. There was invariably an increase in weight during the perfusion, and this increase we took as a measure of the extent of the vascular engorgement and œdema. The average increase in weight amounted to 80 p.c. All calculations of the histamine content were based on the weight of the lung before perfusion. In the extraction of the histamine from the various tissues we employed for lung and kidney the method in which the tissue is treated first with 96 p.c. and then with 60 p.c. alcohol, fats being removed from the extracts by shaking with ether [Best, Dale, Dudley and Thorpe, 1927]. A number of control experiments, in which histamine was added to the lung tissue before it was extracted, showed that the method involved a loss of about 25 p.c. of the histamine, but this loss was constant. In some cases we used the method favoured by Best and McHenry [1931] of disintegrating the tissue with acid. Comparative experiments showed that here the loss of histamine was less, but we prefer the former method, chiefly because of its convenience. Since we invariably worked up a control lung under the same conditions as the perfused lung, the absolute loss of histamine was of no moment. We found that the alcohol method could not be conveniently applied to blood, for which we used, instead, precipitation of the proteins and fats by means of Schenk's reagent. Here again control experiments showed that the loss of histamine occurring in the process was about 25 p.c. The histamine was assayed by the Burn and Dale [1926] method of depression of the blood-pressure of the atropinized cat. In a few cases the anæsthetic was ether alone, but in the majority we used medinal following ether induction.

We have tried other methods of assay, such as the chemical methods of Koessler and Hanke [1919] and of Zimmermann [1930] as well as those employing isolated artery strips, isolated rabbit's ear, isolated guinea-pig intestine as test objects, but we concluded that depression of the cat's blood-pressure was probably the most satisfactory available method. Nevertheless, it is not suitable for the determination of small changes in histamine content. In our previous paper we have quoted 20 p.c. as being the limit of our possible experimental error, but this value certainly varies with different animals. In many cases it is less than 20 p.c., but in a few, at least it is greater.

The average histamine content of the normal lung of the dog, calculated from twenty-four experiments, was found to be 16·9 mg./kg. This

value is lower than that found for cats (36·4 mg./kg.), but individual variations in the dog covered a much smaller range. The histamine content per unit weight of the control lung was taken as indicating the initial histamine content of the perfused lung. The results of five such experiments are summarized in Table I, which shows that perfusion of the intact isolated lung brings about neither inactivation of the histamine present in the lung nor its redistribution between the lung and the defibrinated blood.

TABLE I. Isolated perfused lung.

Exp.	Duration of perfusion, min.	Histamine in the lung, mg./kg. Before perfusion	Histamine in the lung, mg./kg. After perfusion	Histamine change in lung, mg./kg.	Percentage histamine change in blood
1	140	10·2	12·5	+2·3	0·0
2	180	16·7	16·7	0·0	0·0
3	180	25·0	25·0	0·0	0·0
4	180	12·5	12·5	0·0	0·0
5	180	10·0	10·0	0·0	0·0

These experiments also confirmed many others that we have made which all tend to show that defibrinated blood contains no detectable histamine as a normal constituent. On many occasions we have prepared extracts of blood by the alcohol-ether method and by precipitation with Schenk's reagent. We have used fresh whole blood, fresh defibrinated blood, and defibrinated blood which has been incubated for varying periods of time, without ever obtaining evidence of the presence of significant amounts of histamine. Most of these extracts showed a slight depressor action on the blood-pressure of the atropinized cat, equivalent (an average figure) to 1 : 4,000,000 histamine, agreeing with the figure previously given by Harris [1927] for human blood. But that this depressor substance in normal blood is histamine is rendered extremely doubtful by the conclusive demonstration by Zipf [1931] of the normal presence in fresh defibrinated blood of adenylic acid. We found that solutions of adenylic acid, in concentrations equivalent to those found by Zipf for blood, produced depression of the cat's blood-pressure of the same order as we obtained with the blood extracts.

II. PERFUSED KIDNEY-LUNG PREPARATION.

We are now in a position to investigate the effect of including the kidney in the circuit. The perfusion apparatus used for the isolated lung was extended to include the kidney in series. Means were provided for controlling and measuring the pressure in the renal artery and the blood flow through the kidney, the arrangement being somewhat similar to

that used by Hemingway [1931], Bayliss and Lundsgaard [1932]. The transfer of the kidney from the intact animal to the circuit was performed in as short a time as possible, which in the majority of experiments did not exceed 60 sec. We are indebted to Prof. E. B. Verney (personal communication) for the details of the operative technique.

TABLE II. Perfused kidney-lung preparation.

Exp.	Time of perfusion, min.	Histamine in lung, mg./kg. Before perfusion	Histamine in lung, mg./kg. After perfusion	Histamine in kidney, mg./kg. Before perfusion	Histamine in kidney, mg./kg. After perfusion	Histamine change in lung, mg./kg.	Lung blood flow, c.c. per min.	Maximum kidney flow, c.c. per min.
1	240	20·0	33·0	N.T.	N.T.	+13·0	160	114
2	150	16·7	19·4	0·2	0·2	+2·7	240	60
3	180	12·0	12·0	0·5	0·4	0·0	250	155
4	130	19·6	21·8	0·17	0·21	+2·2	400	240
5	180	16·7	16·7	0·4	0·4	0·0	—	70
6	109	30·0	30·0	0·24	N.T.	0·0	250	100
7	132	10·0	10·0	0·2	0·2	0·0	270	112

N.T. = Not taken.

The results of seven experiments in which kidney and lung were perfused together are given by Table II. We estimated the histamine content of the remaining kidney for the purpose of control. The table shows that perfusion for periods varying from 2 to 4 hours does not induce the inactivation of any part of the naturally occurring histamine in the lung or kidney. This histamine is principally present in the lung. As already stated the blood contains no histamine, and columns 5 and 6 show that only a negligible amount is present in the kidney, before or after perfusion. This fact has already been noted [Best and McHenry, 1930]. One experiment only is not in keeping with this general conclusion. In Exp. 1 it is seen that a change occurs in the amount of histamine in the lung. The amount, 65 p.c. is outside our presumed limits of experimental error, but it is to be noted that it is an increase in the amount of histamine in the lung (and therefore in the system lung-kidney-blood) and not a decrease as one would expect. If this result were accepted, it would imply the actual synthesis of histamine during perfusion. It is, however, contradicted by the six experiments following it, and is neglected in drawing the above conclusions.

In view of the results given in Tables I and II it became necessary to test whether the kidney was actually functioning as an inactivator of histamine when the amine was perfused through it, using the isolated perfused kidney-lung preparation as in our previous experiments and adding histamine to the perfusate.

III. Perfused kidney-lung preparation with added histamine.

Table III summarizes the relevant changes occurring when lung and kidney in series are perfused with defibrinated blood to which histamine (10 mg.) is added. It is clear that most of this added histamine was destroyed, although the perfusion never lasted more than 1½ hours. Moreover, estimation of the histamine remaining in the blood at different times after its addition showed that the bulk of the histamine was inactivated within the first 5 min. after its addition.

Table III. Kidney-lung perfusion with added histamine (10 mg.).

Exp.	Time of perfusion after adding histamine, min.	Histamine in lung, mg./kg. Before perfusion	Histamine in lung, mg./kg. After perfusion	Histamine in kidney, mg./kg. Before perfusion	Histamine in kidney, mg./kg. After perfusion	Percentage change of added histamine in blood	Amount of urine in c.c.	Absolute histamine content of urine (mg.)
1	28	20·0	30·1	0·0	1·0	−100·0	102	1·3
2	35	—	—	—	—	—	132	1·7
3	60	11·65	17·45	0·6	0·6	−100·0	82	0·7
4	85	10·0	13·3	—	—	−98·6	75	0·3

By these experiments we were convinced that the histaminase system of the kidney retained, under the conditions of our experiments, its full activity. The amount of histamine added to the blood was about ten times the amount that could have been furnished by lungs of the size we used. Evidently some cause other than that of inactivity of the kidney must be sought to explain the immobility of the natural histamine in the lung.

It will be seen from Table III that there is no loss in the histamine content of the lung. On the contrary, there is, if anything, in each case an increase, which as we shall see is comparable with the increase observed when the lung alone is perfused with blood containing histamine (Table IV). There is, moreover, no such storage of histamine in the kidney, whose histamine content remains negligible in amount. It would appear, therefore, that if any significance be attached to the small increase in histamine content of the lungs in these experiments, it must arise from the retention of the histamine added to the blood.

In one case only, in our kidney perfusions with untreated defibrinated blood, was the formation of urine observed, and in that case the blood was a mixture obtained from three different animals. In all the cases referred to in which urine was excreted by the kidney, this was attained by diluting the blood with approximately one-fifth of its volume of saline. We found

the addition of saline to have very little effect in increasing lung or kidney œdema. The alternative method of producing diuresis by addition of urea to the blood was not tried. In every case histamine, varying in amount from 0·3 to 1·7 mg., was excreted in the urine. The kidney therefore shows a twofold action on histamine, the greater part it inactivates in virtue of its histaminase, the remainder it excretes in the urine.

IV. Isolated perfused lung with added histamine.

In a further series of experiments the lung alone was perfused with defibrinated blood to which histamine was added. The results are summarized in Table IV, from which it is evident that there is a definite

Table IV. Isolated lung perfusion with histamine added.

Exp.	Pressure	Time of perfusion after addition of histamine, min.	Histamine added to the blood, mg.	Histamine in the lung, mg./kg.		Histamine change in lung, mg./kg.	Histamine change in blood, mg./kg.	Percentage histamine change in system
				Before perfusion	After perfusion			
1	50 mm. Hg	40	3·0	18·2	20·0	+1·8	−19·0	−11·7
2	30 ,,	50	3·1	20·0	29·3	+9·3	−45·1	−16·5
3	18 ,,	120	3·94	23·6	33·1	+9·4	−18·0	− 2·6
4	12 ,,	215	2·04	26·7	26·7	0·0	−17·1	− 9·5
5	35 cm. H_2O	120	4·00	11·7	20·0	+8·3	−51·1	−36·6
6	35 ,,	83	5·33	12·5	16·7	+4·2	−40·0	−27·4
7	35 ,,	90	5·33	16·7	25·0	+8·3	−28·4	−18·3

loss of added histamine from the system lung-blood on perfusion for 1½–2 hours. In the first four experiments the extent of the loss of this histamine comes within the limits of experimental error, but it is to be noted that in every case the figures in themselves indicate a loss and not a gain of histamine. Considering all seven experiments together, we feel justified in concluding that the lung perfused with defibrinated blood to which histamine has been added, destroys or inactivates part of this histamine, thus confirming and extending the results obtained by Best with minced lung tissues. A further conclusion to be drawn from Table IV is that, although histamine is destroyed, it appears to be only the histamine which has been added to the blood which is affected. In no case, as shown in column 7, is there a loss of histamine from the lung itself. On the contrary, except in Exp. 4, a small increase in the lung histamine is observed. In most cases the amount can be only partially accounted for by the accumulation of œdematous fluid containing histamine.

Acetylcholine.

In view of the fact that we had already tested [MacGregor and Peat, 1931] the effect of excitation of the parasympathetic nerves to the lungs on their histamine content, we considered it desirable to observe the influence of acetylcholine injections; we performed two experiments. As before the kidney-lung system was perfused with defibrinated blood. Eserine was first added to the blood to delay or inhibit the destruction of acetylcholine by the blood esterase [Engelhart and Loewi, 1930], followed about 10 min. later by acetylcholine bromide (4–5 mg.). Immediately on addition of the acetylcholine, in the two experiments the blood flow from the kidney rose from 100 and 86 c.c. per min. to 166 and 165 c.c. per min. respectively, while the kidney arterial pressure fell from 140 and 140 mm. Hg to 120 and 95 mm. Hg. This observation supports the findings of Hamet [1926] that acetylcholine dilates the kidney vessels. At the same time constriction in the lungs caused a decrease in flow from 176 and 272 c.c. per min. to 136 and 100 c.c. per min. in the two. In this connection Daly and Euler [1932] obtained vaso-constriction with acetylcholine in some of their isolated perfused lung preparations, in others vaso-dilatation occurred. Recovery of both lung and kidney was rapid, and at the end of the perfusion, although a trace of acetylcholine could be detected in the kidney in each case, there was no trace to be found in the lung. Examination of the lung, kidney and blood for histamine showed that blood and kidney contained none and that the amount in the lung (30·0 and 10·0 mg./kg. respectively) remained unchanged. Acetylcholine, therefore, does not cause the liberation of histamine from the lung in amounts determinable by our technique.

Pharmacological action of histamine on lung and kidney.

It may be of interest to record the effects we observed on adding histamine to the defibrinated blood, perfusing either lung alone, or lung and kidney together. As already pointed out, the pulmonary arterial pressure was constant throughout the experiments, and the renal arterial pressure was also maintained constant at 140 mm. Hg, except at those times when histamine was added to the circulation. In Table V are recorded the changes we observed in the renal artery pressure, the renal vein flow and the pulmonary vein flow on adding histamine to the circulation. These results indicate a definite vaso-constrictor action on the lung, while the vessels of the kidney are apparently dilated. Both effects disappear within 30 min.

TABLE V. Effect of histamine on the vessels of the lung and kidney.

Exp.	Histamine added. mg.	Kidney pressure (mm. Hg) Before histamine	Kidney pressure (mm. Hg) After histamine	Kidney flow, c.c. per min. Before	Kidney flow, c.c. per min. After	Lung flow, c.c. per min. Before	Lung flow, c.c. per min. After
1	10·0	150	130	160	164	—	—
2	10·0	140	140	67	100	300	80
3	10·27	140	140	172	172	240	120
4	10·0	140	130	67	86	400	330
5	10·0	138	120	70	116	260	55

The increased resistance to flow through the lungs produced by histamine is in keeping with the observations of previous investigators [Dale and Laidlaw, 1910], but there appears to be no agreed opinion concerning its effect on the renal circulation. Dale and Laidlaw [1910] also observed a decrease of kidney volume in the intact animal, suggesting an active constriction. Manwaring, Monaco and Marino [1922] reported an increased resistance to flow in the isolated kidney of the dog. Dicker [1928] found a decreased venous outflow, while Morimoto [1928] thinks that histamine constricts the renal vessels. On the other hand Ganter and Schretzenmayr [1929] consider that histamine has a dilating effect on the capillaries of the intact kidney of the cat.

SUMMARY.

The relationship between the histaminase system of the lung and kidney of the dog and the naturally occurring histamine of the lung has been investigated by perfusion of the isolated organs with defibrinated blood.

1. Perfusion of the lung alone or of the lung and kidney in series has no effect on the gross amount of histamine in the lung. It is, however, expressly emphasized that the method of assay allows only of the estimation of large changes in the histamine content (20 p.c. or more) and says nothing about changes of a lower order than this.

2. When the lung alone is perfused with defibrinated blood to which histamine is added, part of this added histamine is inactivated, although the naturally occurring histamine in the lung is unaffected.

3. When the lung and kidney are perfused together with defibrinated blood containing histamine, most of the added histamine is rapidly destroyed, while some is excreted in the urine. Again, the natural histamine of the lung is untouched, despite the fact that when ten times the quantity of histamine in the lung is added to the blood, the greater part of this added histamine is inactivated within 5 min.

It is concluded therefore that within the limits of our experimental methods, as defined above, there appears to be no simple humoral connection in the perfused organs between the histamine of the lung and the histamine of either the lung itself or of the kidney.

We are greatly indebted to Prof. I. de Burgh Daly for his helpful criticism and advice.

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THE CENTRAL AND THE REFLEX MECHANISM OF PANTING.

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Richet [1898], when describing the effect of exposure to heat on respiration in dogs, pointed out the striking difference between the behaviour of anæsthetized and non-anæsthetized animals. Non-anæsthetized dogs pant without a rise of body temperature, by simply being exposed to the sun for 2–10 min. To induce panting after administration of an anæsthetic (Richet used chloralose), however, the body temperature of the animal must be raised appreciably above normal. Richet assumes that in the first case the panting is based on a reflex, initiated by warming the terminations of the cutaneous nerves and of the fifth cranial nerve, while in the second panting is the direct result of raising the temperature of the brain centres.

A previous paper, published jointly with Prof. G. V. Anrep [Anrep and Hammouda, 1932], dealt with the respiratory changes which take place in the animal during panting. In the present communication it is proposed to study the nervous mechanism underlying the phenomenon of panting.

Richet thought that this peculiar form of respiratory activity was governed by the medulla. But subsequent researches on the Sherrington decerebrate preparation show that the medulla cannot be responsible for panting, since, in decerebrate dogs and cats, even at very high body temperatures, the highest respiratory rate does not exceed 60–70 per min.; decerebrate animals do not pant. Nikolaides and Dontas [1911] found that heat polypnœa cannot occur if the medulla is separated from the brain. They describe a heat polypnœa centre in the corpus striatum. Bazett and Penfield [1922] found in acute decerebrate cats and dogs a maximum rate of 60 at a temperature of 42·8° C., and Sherrington [1924] found in acute decerebrate cats and dogs a maximum rate of 75 with a rectal temperature of 41·1° C. On comparing the maximum rate of 60–75 per min. obtained in these decerebrate preparations with the

maximum of 300–400 observed in anæsthetized panting animals, one is led to the conclusion that the parts of the central nervous system responsible for the very high rates of respiration lie above the mid-brain.

The "panting centre."

The following experiments were carried out in order to try to localize more precisely the part of the central nervous system which is responsible for panting. It was hoped that by removal of various parts of the cerebral hemispheres it would be possible to locate, at least with some probability, that part of the brain which, when destroyed, interferes permanently with panting. Two different procedures were adopted. In the first, the section of the brain was performed beforehand (*i.e.* before panting); the dog was left to recover from the anæsthetic and from the immediate effect of the operation, and then warmed up. In the second, the brain was freely exposed on one or both sides, depending on the section to be performed; the animal was then warmed up until panting became definitely established, and the section of the brain was made during the progress of panting. When the first procedure was adopted, the dogs were operated upon under chloroform and ether anæsthesia; in the second case they were given chloralose intravenously (0·05–0·075 g. per kg.). All these animals were supplied with a tracheal cannula and had both carotid arteries tied. They were warmed up either in a hot bath at 40–42° C., or in an air thermostat by a method described in a previous communication [Anrep and Hammouda, 1932]. The respiratory rate was recorded by a stethograph. After the end of an experiment, the brain was hardened *in situ* with formalin; it was then removed from the skull and carefully examined to determine the extent of the injury[1]. The operations on the brain were all bilateral. The experiments with the section of the brain at different levels can be classified under the following four headings:

(1) Removal of the cortex with preservation of the basal ganglia.

(2) Removal of the two hemispheres leaving the mid-brain intact.

(3) Removal of the cortex and of the corpora striata without damaging the optic thalami.

(4) Local injuries to the optic thalami.

Fig. 1 shows the levels at which these sections were made.

In the first group, the animals were decorticated on both sides by scooping out the brain substance, care being taken not to injure the basal

[1] I am greatly indebted to Dr B. Boulgakow, of the Anatomy Department, for the help he gave me by making the post-mortem examinations of all the brains.

ganglia; the cavity of the skull was then lightly packed with cotton-wool soaked in warm saline. The post-mortem examinations of these brains showed that the basal ganglia were completely intact, and that practically the whole cortex had been removed, *i.e.* only occasionally had small scraps of the cortex been left. All these animals reacted to the rise of body temperature by panting as vigorously as normal animals. This was observed in dogs in which the cortex was removed beforehand, as well as in dogs which were decorticated during the progress of panting. In the latter case, the panting respiration was slightly disturbed during the

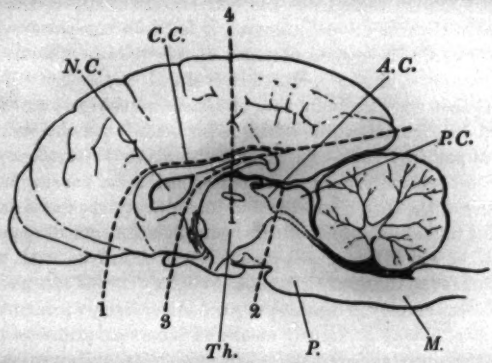

Fig. 1. Sagittal section of dog's brain, demonstrating the operations (1, 2, 3, 4) performed. *C.C.* corpus callosum; *Th.* optic thalamus; *N.C.* nucleus caudatus; *A.C.* anterior colliculus; *P.C.* posterior colliculus; *P.* pons; *M.* medulla. Further description is given in the text.

actual surgical interference, but afterwards it continued without interruption. Panting as a response to raising the body temperature is thus shown to be independent of the cortex.

In the second group of dogs, the section was made at the level of the anterior colliculi. The post-mortem examinations showed that the brain stem was completely severed in all cases; no parts of the optic thalami had been left behind. In a few experiments it was also found that the anterior colliculi had been destroyed, but the posterior colliculi remained uninjured in every experiment. During the operation on non-panting dogs, the respiration stopped for a moment and then resumed its original rhythm. On warming up these animals, the respiration accelerated only to a maximum of 40–60 per min. with a rise of rectal temperature to

42·0° C. The higher respiratory rates were observed in those dogs in which the plane of section was more anterior, thus causing less disturbance to the mid-brain. When the decerebration was made in a panting animal, the respiration stopped during the section and then resumed at a slow rate, which gradually rose to a maximum of 40–60 per min. In two of these experiments, the temperature was raised until the dogs succumbed to heat; panting, however, never took place. Before death, the respiration became slow and was occasionally interrupted by gasps. In the other experiments, the animals were allowed to cool. It was noticed that in these cases the temperature dropped considerably more rapidly than in normal animals; it fell in a comparatively short time to 33–31° C. The results obtained on decerebrate animals confirm the observations of Bazett and Penfield [1922] and of Sherrington [1924] that the maximum rate obtained in such preparations does not exceed 75 per min., a rate which is far below even the slowest rate obtained in panting. These experiments also make it clear that the part of the central nervous system which is responsible for panting lies somewhere between the cortex and the mid-brain, *i.e.* in the basal ganglia.

In order to find out which of these ganglia is concerned, experiments were made in which the corpus striatum was destroyed with the least possible damage to the optic thalamus. A slightly curved spatula, 1·3 cm. in breadth, was employed for this purpose. After freely opening the skull on both sides, having previously measured the exact distances by comparison with a preserved brain of the same size, the spatula was introduced through the occipital lobe. It was held so that its lateral edge was somewhat lower than the other edge. The spatula was pushed into the brain substance in this slanting position until the anterior limits of the thalami were passed, and then it was pressed downwards to the base of the skull. The operation was repeated on the other side of the brain. As a result of this operation it was hoped to destroy the greater part of the corpora striata without injuring the optic thalami. Three experiments were successfully performed. The post-mortem examinations of these animals, made by serial frontal sections, showed that the lenticular and the caudate nuclei as well as the internal and the external capsulae were in every case destroyed almost completely; the colliculi and the mid-brain were intact. The optic thalami were intact in two cases, while in the third their anterior parts were slightly bruised. Of the two dogs with the intact thalami, one was operated upon during panting; its respiratory rate was 200 per min. and its rectal temperature 41·4° C. The respiration was momentarily interrupted during the sections, but

soon after the operation the dog was breathing at 60 per min., and within 1 min. it was again panting at the rate of 200. The animal was then cooled; at a rectal temperature of 40·7° C. panting disappeared, the respiratory rate being 40 per min. The dog was cooled down to 37° C. and, on rewarming, it began to pant at the rate of 200–250 per min. when the rectal temperature reached 40° C. The second dog, which was operated upon 3 hours before the exposure to heat, panted at 120 per min. with a rectal temperature of 40·5° C., and at 250 per min. with a temperature of 41° C. In the third dog also the operation was performed 3 hours before warming. On exposing this dog to heat, its rectal temperature gradually rose to 42·5° C., and the respiration accelerated with the rise of temperature to 58 per min.; the breathing then became irregular and was interrupted by deep gasps. No panting was observed. The dog was cooled and then warmed up to 43° C. with exactly the same result. This was the dog in which the thalami were found to be injured.

These experiments with acute spino-thalamic animals show that the development and the extent of panting is of the same character as that observed in normal animals, and that the least injury to the thalami abolishes panting and makes the animal behave as if it were decerebrated. As in all the three experiments the corpora striata were almost completely destroyed one is led to the conclusion that these ganglia are not concerned in panting. The experiments strongly suggest that the part of the central nervous system on which panting depends, or to give it a name the "*panting-centre*," is located in the optic thalamus. This conclusion is supported by experiments in which four or five serial frontal sections of the brain were made during the progress of panting, and by experiments in which localized injuries to the thalami were inflicted. The roof of the skull was completely removed, the longitudinal sinus was removed between ligatures, and the brain was exposed. The dog was then kept immersed up to its neck in a hot bath, until panting became well established. With a sharp flat spatula, frontal sections were made across the hemispheres down to the base of the skull. The first section was made through the anterior parts of the frontal lobes; the panting was undisturbed. After an interval of a few minutes, a new section was made about 1 cm. behind the preceding section; this also had no effect. Sections were continued at short distances behind each other until panting disappeared, after which no more sections were made. In the three experiments in which this was carried out, panting remained unaffected until a section passed through the optic thalami. It should be noted that a section which passes just in front of the thalami definitely

cuts through the heads of the caudate nuclei. Such a section, however, has no permanent effect on panting.

Localized injuries to the thalami were made by means of a small metal tube. During the progress of panting, the tube was introduced between the hemispheres, through the corpus callosum and the fornix into the third ventricle. This in itself had no effect on panting. The thalami were then injured on both sides, and the extent of the injury was determined in the post-mortem examination of the brain. Four such experiments were made. During the actual injury, the respiration either stopped or showed considerable irregularities. After removal of the metal tube these irregularities persisted for a short time, but soon the respiration assumed a regular rate which varied in different experiments from 60 to 100 per min. When the warming of these animals was continued, the respiration rate did not increase any further. In a few experiments, the damage of the thalami was made by cauterization with a fine electro-cautery, which was passed into the third ventricle in the same manner as the metal tube. It was usually found that the greater the damage to the optic thalami, the slower was the respiration; but even comparatively small amounts of damage may considerably reduce the rate of panting. Fig. 2 shows the extent of the damage to the optic thalami in two cases. The results of serial sections of the brain and of local injuries to the thalami thus definitely indicate that panting is dependent on the integrity of the optic thalami.

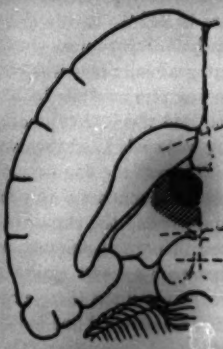

Fig. 2. Diagram to illustrate the localized injuries to the thalamus, as found in the post-mortem performed after the two experiments referred to in the text. The extent of the injuries in the two experiments is represented by the boundaries of the two differently shaded areas. In each case the injury was almost identical on both sides of the brain. *Th.* optic thalamus; *N.C.* caudate nucleus; *A.C.* anterior colliculus.

Abundant evidence is given in the literature that the temperature control of the animal is situated above the level of the mid-brain. Barbour [1912] locates the thermotaxic centre in the corpus striatum, but Cloetta and Waser [1914] do not support this view. Isenschmid and Krehl [1912], Isenschmid and Schnitzler [1914], Leschke [1913], de Barenne [1920], and Bazett and Penfield [1922] are of the opinion that the actual position of the centre which controls body tem-

perature is probably in the thalamus, hypothalamus or tuber cinereum. Panting is a specialized mechanism of regulation of the body temperature. It is therefore not suprising that the centre for panting should be localized in the same region as the other temperature controlling centres.

The central mechanism of panting.

Raising the body temperature of an anæsthetized dog leads to panting. Richet ascribed this to a direct stimulation of the respiratory centre by heat, but he advanced no conclusive evidence in favour of this view. Richet refers to the experiments of some older observers who found that panting can be produced by warming the carotid blood supply. These observations were confirmed by Kahn [1904] and by Heymans and Heymans [1926]. Bazett [1927], however, rightly remarks that the experiments in which the carotid blood stream is warmed cannot be regarded as conclusive evidence of the direct stimulation of the centres by heat, unless precautions are taken to exclude the influence of sensory impulses through the cranial nerves. This criticism assumes a special importance since Richet points out that the application of heat to the terminations of the fifth cranial nerve plays an important part in the production of panting in non-anæsthetized animals.

The following experiments were performed in order to determine whether the panting produced by warming the carotid blood is really due to a direct heat effect on the centre. The vertebral arteries were tied and the animal was heparinized. The central ends of both common carotid arteries were connected with a glass spiral which was immersed in a water bath. The blood flowing through the spiral (where it could be warmed or cooled) was led into the peripheral ends of the common or of the internal carotid arteries. The temperature of the blood was measured close to the peripheral ends of the arteries. The venous blood was allowed to return to the heart of the animal. The temperature of the body, which was measured in the rectum, was kept as constant as possible. On warming the carotid blood, panting could be obtained with great ease. No difference was observed between the panting of animals which were perfused through the internal or the common carotid arteries, showing that the presence of the carotid sinus plays no rôle in panting. This was confirmed by two experiments in which the whole animal was warmed after extirpation of the carotid sinus on both sides. The panting presented no difference from that in animals with intact carotid sinus. These results are in agreement with the observations of Heymans and Heymans [1926] and Tournade and Malméjac [1930].

In the experiments in which the head is perfused through the internal carotid arteries (the vertebrals are tied) besides the exclusion of the carotid sinus, the blood supply to the peripheral structures of the head and therefore to the terminations of the fifth cranial nerve must be considerably reduced. In order to test whether panting may be caused by warming these or other sensory terminations in the head, the following additional observations were made. The head of an animal, while being perfused through the internal carotid arteries by a blood supply sufficiently warmed to cause panting, was surrounded by ice, and the nasal and the buccal cavities were continually irrigated with cold saline. The panting, however, remained unchanged. Further, it may be added that the removal of the whole skin of the head also fails to affect panting. These experiments show that at least the external sensory terminations of the head play no rôle in the causation of panting in response to raising the temperature of the blood. It has already been mentioned that extensively opening the skull and the meninges, which necessarily prevents their warming, does not modify panting. Unless there are specialized heat receptors in the meninges at the base of the brain or in the cerebral blood vessels, all the evidence points to panting as being produced by an entirely central mechanism, *i.e.* by the direct effect of heat on the "panting centre."

In several experiments an attempt was made to produce panting by direct application of heat to the optic thalami. A narrow Kronecker's cannula was introduced into the third ventricle by the technique described above. Great care was taken to fix the cannula in such a position that it should not exert pressure upon the thalami. One branch of the cannula was connected with a reservoir containing warm saline, and the other was connected with a rubber tube which served as an outlet. The third ventricle was perfused at a low pressure with warm saline. The temperature was measured in the reservoir and in the inlet branch of the cannula. During the irrigation of the ventricle with saline at 38° C., the respiration was regular throughout. On raising the temperature of the perfusing fluid in the inlet cannula to 42° C., it was observed in some cases that the animal started to pant. The panting could easily be stopped by dropping the temperature of the perfusing fluid. The panting rate was in no case very high, but, as can be shown by a method described below, it was a genuine panting and not a simple acceleration of the ordinary respiration. Warming of the thalami by this method would obviously raise the temperature chiefly at the surface of the thalami, without much affecting the rest of the thalamus; moreover, manipu-

lations in this region would invariably cause some disturbance or damage. This is probably the reason why local warming of the thalami never leads to such high rates of panting as are observed in the case of warming the carotid blood or the whole animal. In fact, in experiments in which the introduction of the cannula caused definite damage to the thalami, perfusion with hot saline failed to induce panting. A macroscopic examination of the thalami was made after the end of each experiment. It must be specially emphasized that experiments with the direct warming of the thalamus are not invariably successful, but those which were successful leave no doubt about their result. It is important to choose a suitable anæsthetic. In chloralosed animals, the effect was not obtained. Urethane or alcohol anæsthesia was found to be the best. It is of interest to mention that in some experiments a gentle mechanical stimulation of the mesial surfaces of the thalami frequently produced panting, but the rate did not exceed 120–130 per min. The following is an experiment with direct warming of the thalami.

Dog 6 kg., morphine 25 mg., 5 c.c. of 42 p.c. alcohol intravenously. The injection of alcohol was repeated in 1 c.c. doses during the experiment. The roof of the skull was removed, the meninges opened and the longitudinal sinus cut out between ligatures. All bleeding points were stopped. The respiratory rate was 22 per min. 30 min. after opening the skull, a thin Kronecker's cannula was introduced into the third ventricle. The respiration at first increased to 34, and then returned to 22 per min. Saline at 38° C. was now passed through the cannula into the ventricle; the outflowing fluid at first contained traces of blood, but in a few minutes it became clear. The respiratory rate fluctuated between 20 and 22. The perfusion was now changed to saline at 42° C. (registered in the inlet of the cannula). The following were the respiratory rates during successive 20 sec., starting from the moment of the irrigation with hot saline; 20, 60, 80, 80, 100, 100, 120, 120, 120, 130, 130, 130 per min. The ventricle was now perfused with cold saline at 25° C. The rates were 100, 80, 60, 60, 50, 48, 36, 22, 18 per min.

This experiment was repeated several times on the same animal with the same result. The cannula was then introduced more deeply into the third ventricle, and moved in a frontal plane so as to produce localized damage to the thalami. The irrigation was continued. The respiration, after some irregularities, returned to 18 per min. and remained at this rate. Irrigation of the ventricle with hot and cold saline now produced no change in the respiratory rate. The rectal temperature of the animal was kept between 37·0 and 37·5° C. throughout the experiment. The post-mortem examination showed extensive damage of the mesial parts of both thalami; the corpora striata and the anterior colliculi were intact.

The reflex mechanism of panting.

The experiments with perfusion of the carotid arteries, and with the direct application of heat to the optic thalamus, are strong evidence in favour of the existence of a central mechanism of panting. Richet assumed that this was the only mechanism on which panting depends, in animals which have been subjected to anæsthesia. He did not, however, perform any experiments in order to verify this assumption. The question whether there is also a reflex mechanism, which co-operates with the central one, still therefore remains unanswered. In other words, we do not know whether warming of some sensory terminations of the body in an anæsthetized animal may evoke reflex panting. It would be legitimate to suppose that the skin of the animal would be the most likely organ to act as a receptor in such a reflex. This is, however, not the case, as a dog will pant when its body is warmed even after the removal of the whole skin. The body temperature at which panting develops does not differ from the panting temperature of a dog whose skin is intact. The rate of panting is also of the same order. The following experiment is an example.

Dog 3·5 kg., morphine 20 mg., chloralose 0·25 g. On exposure to heat in a water bath at 42° C., the dog began to pant at a rectal temperature of 40·0° C. When its temperature rose to 40·5° C., the dog panted at a regular rate of 200. The dog was cooled and the anæsthesia was deepened by administration of chloroform and ether mixture. The whole skin of the animal from the tip of the nose to the end of the tail was then removed, care being taken to stop all bleeding points. The volatile anæsthetic was discontinued, and the animal was at once placed in a saline bath at 38° C. and allowed to rest for 45 min., after which the temperature of the bath was raised to 41° C. The dog, as before, started panting at a rectal temperature of 40·0° C. and soon reached a rate of 230 per min. with a rectal temperature of 40·2° C.

This experiment definitely shows that the skin plays no rôle in panting produced in response to the warming of the whole body. The following experiments, in which the carotid blood stream was warmed or cooled independently of the changes of the body temperature, demonstrate further that the warming of the internal organs of the dog also does not cause panting.

The blood stream in the common carotid arteries was passed through a water bath, in the same manner as in the experiments referred to before, while the body of the dog was placed in an air thermostat. The temperature of the head was measured by a thermometer placed deeply under the tongue, and the temperature of the body by a thermometer in the rectum. In the first part of the experiment, the body and the carotid blood were warmed at the same rate. The animal, which breathed at the rate of 18–22 per min., gradually increased the respiration to 33 per min. At a temperature of 41° C. in the mouth

and rectum the animal abruptly began to pant, increasing its panting within 4 min. to 240 per min. The head was now gradually cooled to 38·5° C., but the body was warmed still further until the rectal temperature reached 42·5° C. In spite of this, the respiration dropped to 26 per min. On rewarming the carotid blood to 41° C., the panting returned to 250 per min. The head was then kept at 41° C., while the whole body was gradually cooled to 37·5° C. The panting rate did not change; it fluctuated between 230 and 250 per min.

A second experiment was started by warming the body alone, the mouth temperature being kept at 37·5° C. The rectal temperature was gradually raised to 41·5° C. but the respiration remained unchanged; it varied between 26 and 30 per min. The body was then cooled to 37° C. while the head was gradually warmed; at a mouth temperature of 39° C., the respiratory rate became 200 and, at 39·5° C., 290 per min.

These experiments give strong support to Richet's statement that in chloralosed dogs panting is entirely of central origin. Reflex panting could not be observed in anæsthetized dogs.

Respiratory reflexes during panting.

Richet [1898] refers to Goldstein and Sihler as having been the first to show that section of the vagi does not affect panting. This observation was verified and extended in a previous communication [Anrep and Hammouda, 1932]. It can now be added that in panting, besides playing no rôle, the vagus nerve also loses its normal controlling action of the respiratory movements. The reflex arrest of the respiration in response to inflation of the lung [Hering and Breuer, 1868] is absent during panting (see Figs. 3 and 4). This could be seen in every experiment. It is so constant a phenomenon that it is convenient to take the disappearance of the reflex to inflation as an arbitrary criterion for distinguishing between panting and fast rates of normal respiration. Usually the conspicuous difference in the rate between ordinary respiration and panting leaves no doubt as to which type of respiration one is dealing with. But in some cases, especially in deeply anæsthetized animals in which the panting rate may not exceed about 120 per min., the disappearance of the reflex is of considerable help. The disappearance of the pulmonary vagal reflex is not abrupt. During the period of transition to panting, inflation of the lungs can still produce a brief arrest or slowing down of the respiration. This arrest, however, differs from that observed in the case of ordinary respiration by being considerably delayed in its appearance. The normal respiration is stopped at once as soon as the lungs are inflated, while in panting there are always a few respiratory movements before the arrest; these respiratory movements are frequently unchanged in rate (see Fig. 4*a* and *b*). As panting develops, progressively larger degrees of inflation are required in order to produce this delayed

pause. Finally, as panting becomes established, inflation of the lungs ceases to have any inhibitory effect on respiration. In several experiments, an acceleration of the respiration was noticed during the inflation, instead of an inhibition. Although gradual, the disappearance of this Hering-Breuer reflex occurs rapidly. In most experiments, the transition period lasts approximately the same length of time as it takes the dog to develop the fast rate of respiration which is definitely recognized as panting. This may require from 10–15 sec. to 1–2 min.

Fig. 3. Effect of deflation and inflation of the lungs during the transition from normal respiration to panting. The lungs were deflated and inflated by positive and negative pressures applied to the animal which was enclosed in a box. *A*. Effect of deflation by +11 mm. Hg at a respiratory rate of 80 per min. The respiration accelerated to 100 per min. *B*. Effect of inflation by −9 mm. Hg at a respiratory rate of 95 per min. Read from left to right. Time in 10 sec.

In the great majority of the experiments (over 200 dogs), the respiratory rate of 120 per min. could be recognized as an unquestionably established panting. Rates between 75 and 120 showed a progressive diminution, but not a complete absence, of the respiratory response to inflation of the lungs.

The usual acceleration of the respiration in response to a deflation of the lungs [Hammouda and Wilson, 1932] could not be observed during panting. For instance, in one experiment, just before the development of panting, the animal's respiration, which was 54 per min., accelerated

to 108 when its chest was exposed to an outside positive pressure of 13 mm. Hg. A few minutes later the animal started panting at the rate of 120. The compression of the chest by 16 mm. Hg air pressure did not

Fig. 4. Effect of inflation and deflation of the lungs during panting, from the same dog as Fig. 3. *A*, *B* and *C* show the effect of inflation (−8 mm. Hg); the respiratory rates were 114, 120 and 150 respectively. In *D*, deflation (+9 mm. Hg) did not change the rate of 162 per min. Time in 10 sec. Read from left to right. The line under each respiratory record shows the pressure change in the box enclosing the animal. The respiration was recorded by means of a bellows recorder which was attached to a closed respiratory system of 20 litres capacity, connected with the trachea of the animal. In between the records, the trachea of the dog was opened to the air.

change the rate. It is obvious that these experiments are not as conclusive as those with the inflation of the lungs, since it is always possible to assume that the respiration was already too fast to be further accelerated by the deflation of the lungs.

The absence of the Hering-Breuer reflex is not due to a possible effect of heat upon the terminations of the vagus nerve in the lungs. This is seen from experiments in which panting was produced by warming the carotid blood while the body temperature was not raised; in this case inflation and deflation of the lungs also failed to change the rate of panting.

Panting in the normal dog.

On comparing panting in anæsthetized and non-anæsthetized animals, one is at once struck with the fact that, while in the former panting is undoubtedly of a central nature, in the latter it exhibits all the properties of a reflex. This contrast is still further accentuated by the fact that, under ordinary experimental conditions, it seems impossible to obtain reflex panting in the anæsthetized dog, while in the normal dog it takes place with the greatest ease. A dog which is exposed to the sun begins to pant in a few minutes. Careful measurements of the rectal temperature in most cases show an initial rise of about 0·1° C. This rise of temperature cannot, however, be the cause of panting since, as soon as panting starts, the temperature drops below the prepanting level, usually not less than 0·3–0·5° C. and sometimes as much as a whole degree. This was observed by Richet and used by him as a proof of the purely reflex character of panting in non-anæsthetized animals.

It can easily be shown that not only exposure of the whole animal to the sun but also exposure of parts of its surface leads to panting. For instance on one occasion, when the head of a dog was exposed to the sun while its body was kept in the shade, panting started in 5½ min. On exposing the hind quarters it started in 6 min., while exposure of the whole animal evoked panting in 3 min. The temperature of the sun in this case was only 46° C.[1] On another occasion on exposing the head alone, when the temperature in the sun was 52° C., panting started in 5½ min., while on exposing the body with the head kept in the shade it started in 30 sec. At a still higher temperature (65° C.), the dog may start panting almost at once. No precise determinations were made of the minimal surface which had to be exposed to the sun in order to obtain panting. It became evident, however, from the observations made that the exposure of the head does not play any special rôle in com-

[1] The monthly mean temperature in Cairo, taken with the black bulb thermometer, at 2 p.m. in the sun, varies between 51° C. in December and 68° C. from June to October. The relative humidity is 62–66 p.c. during winter and 45–55 p.c. from February to September.

parison with the rest of the body. The rectal temperature of these animals either remained unchanged or rose not more than 0·1° C.

Richet states that, in the conscious animal, panting does not take place at all if some obstruction to respiration is introduced. For instance, he quotes as an example that a muzzled dog does not pant. As a consequence of this, its body temperature rises in a short time. This inhibitory action (of closing the mouth) on panting is, however, far from being so simple. Muzzling a dog for the first time may delay the appearance of panting for half an hour or more, and the animal's temperature may rise quite appreciably; but in the end panting invariably starts. Placing the muzzle on an already panting animal may stop panting completely for many minutes. It is of importance that if the muzzle is used for the second or third time it loses its inhibitory effect. An animal which is accustomed to the muzzle begins to pant almost as soon as without the muzzle; muzzling such an animal while panting is in progress does not stop panting any more. There is, however, one effect of closure of the mouth which remains. With the muzzle, the panting is slower and deeper than without. For example, placing the muzzle on an animal, which was already comparatively accustomed to it, reduced the panting rate from 300 to 220 per min.; on removing the muzzle, it returned to 300. It has not yet been found whether this effect is constant or shows signs of diminution on repetition of the experiments. The abolition of panting observed during the first application of the muzzle is not due to closure of the mouth. In fact, any other discomfort may also produce arrest of panting. A slap, rapid lifting of the animal, or the application of any other stimulus, if sufficiently strong, abolishes panting for some time. Auditory and strong visual stimuli may do the same. All these stimuli are alike in that their repetition rapidly ceases to have an effect. In this respect these stimuli show a striking similarity to the well-known phenomenon of external inhibition of conditioned reflexes [Pavlov, 1927].

It is not only external stimuli which may stop panting. If the animal is thirsty or hungry, if its bladder or bowels are full, exposure to sun does not cause panting until its rectal temperature rises appreciably above normal, which usually takes 30–45 min. at an external temperature in the sun of 37–45° C. When the cause of the discomfort is removed, exposure to the sun again produces panting within a very short time, and without a rise of body temperature. In many cases the disturbing cause could not be traced.

The similarity between panting in the non-anæsthetized animal with conditioned reflexes was so striking that it seemed probable that what is

known as reflex panting is really a conditioned reflex. It is as yet impossible to state whether every case of reflex panting, occurring without or with a minimal rise of body temperature, is conditioned panting. But the failure to obtain panting in anæsthetized animals lends support to this supposition.

Conditioned panting can be established with great ease. In several animals this was done in the following manner. A dog, which was quite new to this type of experiment but accustomed to the laboratory, was placed in a specially constructed large box, where it was subjected to a high temperature. The box was well ventilated. Ten shielded carbon-filament lamps were used as a source of heat, and the temperature of the box was kept at 45° C. In every case the respiration was recorded graphically by means of a stethograph. The development of conditioned panting will be described in relation to one dog only. On several days before the experiment the dog was placed in the unheated box (20–22° C.). In no case did it start panting. The dog was then placed in the heated box once or twice a day. The latent period during the first six exposures varied between 8 and 4 min., gradually becoming shorter. On the third week of working with this animal, the latent period shortened to between 30 sec. and 2 min. On several occasions the dog panted as soon as it was placed in the box. The experiment would usually be terminated a few minutes after the onset of panting. The maximum panting rates were between 150 and 200 per min. After forty-one exposures, the dog was placed in the box which was not heated (20° C.). The respiration of the animal was 22 per min. Thirty seconds after the introduction into the cold box the respiration increased to 60–80, and in the following minute to 120. The second test was made the next week, after a few more reinforcements of the reflex. The latent period was again 30 sec. During several trials, in between which the reflex was steadily reinforced every day, the latent period was found to vary between 15 and 30 sec. The maximum rate of this conditioned panting was between 120 and 150 per min.

Conditioned panting undergoes experimental extinction as can be seen from the following experiment. On the third month of the development of the reflex, the dog was placed in the cold box and then removed about 30 sec. after the onset of panting. After an interval of 5 min. the dog was again introduced into the box; this time the latent period was considerably longer. The procedure was repeated until panting could not be obtained, which happened on the fifth introduction into the cold box.

The phenomenon of external inhibition could also be easily observed. This effect on conditioned panting is remarkably similar to the effect of extraneous stimuli on a dog which pants under ordinary circumstances when exposed to the sun.

Summary.

1. Serial sections of the brain and localized injuries to the optic thalami made in acute experiments demonstrate that panting takes place only when the optic thalami are intact.

2. Removal of the cortex and almost complete destruction of the corpora striata does not abolish panting.

3. Panting observed in anæsthetized animals on raising the body temperature is due to the direct effect of heat upon the optic thalami. It is not due to any reflexes in response to raising the temperature of sensory nerve endings in the head.

4. Heat directly applied to the third ventricle was, in several experiments, successful in evoking panting. It failed to have this effect after injury to the thalami.

5. The inhibition of the respiratory movements caused by the inflation of the lungs, and the acceleration of the respiration caused by deflation, is not observed during panting.

6. The experiments failed to demonstrate the existence of any special receptors in the body which would reflexly evoke panting in response to a rise in body temperature.

7. The normal non-anæsthetized animal pants reflexly without or with a very small initial rise of temperature. This panting exhibits the characters of a conditioned reflex. Conditioned panting can easily be established experimentally.

8. Spontaneous panting on exposure to the sun and conditioned panting are similar in that they are both subject to external inhibition. Conditioned panting undergoes experimental extinction.

9. It is suggested that what is known as reflex panting is a conditioned reflex which is developed, not on the bases of an unconditioned reflex, but on the bases of the purely central effect of heat. In this respect it would be similar to the conditioned reflexes established as the result of injection of various centrally acting drugs.

The research described in this communication was inspired by some observations made during my conjoint work with my teacher, Prof. W. H. Wilson. I wish to express my grateful thanks for his guidance and

encouragement. The actual experiments were made under the direction of Prof. G. V. Anrep, F.R.S., to whom I am grateful for his constant advice and criticism.

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LAPICQUE'S THEORY OF CURARIZATION.

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Curare will bring about a condition in which an impulse will not pass from nerve to muscle though each tissue will still conduct. This condition is called curarization and the term is extended to include similar states brought about by various drugs and fatigue, etc. Thus "curarization" as used in the present paper will not necessarily signify that the conduction block was produced by curare, but it will simply mean that indirect excitation of muscle has been abolished though nerve and muscle are individually still irritable and able to conduct.

Claude Bernard's analysis of the action of curare is classical, and until the beginning of the present century it was universally accepted that there was a special substance intermediate between muscle and nerve which was particularly susceptible to curare and other drugs. In 1906, however, Lapicque put forward an alternative hypothesis which has subsequently gained great support, so that at present it is very widely held. According to Lapicque the strength-duration curve of a muscle is normally identical in time relations with that of its motor nerve, and this "isochronism" is considered a necessary condition for the propagation of impulses from one tissue to another. If a drug or other agent causes the chronaxie of the two tissues to diverge by more than a ratio of 2:1 then conduction between the two is supposed to become impossible. This theory has not been developed deductively from the physics of the conduction of impulses, and no great weight has been attached to theoretical attempts to explain why conduction should fail when two tissues develop a heterochronism in the ratio 2:1. Essentially, the theory is the expression of experimental observation, and hence its validity rests upon the soundness of the experiments adduced to support it. These experiments, which appear to be of three kinds, are each open to certain objections,

and the aim of the present paper is to provide a critical and experimental study of them in order better to appreciate the basis of Lapicque's theory.

Within the last two years Lapicque [1931] has insisted that the name "Chronaxie" be restricted to very special conditions, hence in this paper Lucas's term "Excitation Time" will be used, for in this way no theoretical distinctions will be introduced into a purely experimental study. The question will be resumed in the Discussion.

Lapicque's first argument.

Historical.

At first sight it would seem that experimentally there could be no doubt as to whether the curarization was or was not accompanied by a heterochronism of more than 2:1. Curare is admitted on all hands not to affect nerve, thus it merely remains to excite muscle directly and compare the strength-duration curves before and after curarization. This is in fact what Lapicque did, but the results are complicated by the difficulty of knowing whether the tissue initially excited was the muscle or the intramuscular nerve twigs. Lapicque used the gastrocnemius muscle which has no part that can safely be regarded as nerve-free, he took no special precautions to avoid stimulating nerves, he obtained strength-duration curves identical with nerve, and found a sudden change in the curve at the moment when indirect excitation was abolished [Lapicque, 1926, p. 245].

It is difficult to reconcile the statement at the bottom of p. 244 with that on the middle of p. 245 referred to above. The first reads "On n'obtient ni changement brusque, montrant qu'on passe de l'excitabilité nerveuse à l'excitabilité musculaire, ni..."; the second "...il est presque impossible de saisir un intermédiaire entre la valeur primitive de la chronaxie, et la valeur double." Then follows a statement of Lapicque's opinion that he was initially stimulating intramuscular nerves.

It therefore appears very likely that initially Lapicque was measuring the strength-duration curve of nerve, and his experiments give no direct information as to whether there is normally isochronism between muscle and its nerve or not. This difficulty is well appreciated by Lapicque, who attempts to support his case by the following argument.

After indirect excitation has been abolished there is no question but that muscle is the tissue investigated. But Lapicque observes that at the moment when indirect excitation fails the excitation time of muscle is double that of nerve, and owing to the continued action of the drug it gets longer and longer. It is therefore argued on grounds of continuity

that before curarization the excitation time must have been within the 2:1 ratio. This conclusion, however, is not a necessary one, for it is at least conceivable that the action of curare on muscle might be like that of veratrine on nerve concerning which Lapicque states [1926, p. 276] that the excitation time first diminishes, and then increases again to normal, curarization occurring in this latter phase. There is nothing in Lapicque's experiments to exclude the possibility that the final excitation time of a curarized muscle is the same as the obscured initial value (or even shorter), for in no experiment so far as I know is any satisfactory evidence produced that the excitation time of muscle and nerve are normally identical.

But quite apart from the above criticism there are two others of much greater weight. Suppose it be granted that in Lapicque's experiment the excitation time of muscle was initially near that of nerve, and finally very different, does it follow that the conduction block was due to this change? It will be shown that curarization may occur without any change of excitation time and again that the excitation time may change without curarization, that in fact this change when it occurs is merely incidental and in no way the basis of the conduction block.

Almost as soon as Lapicque's theory appeared his experiments were repeated by Boehm [1910] in a very thorough investigation which failed to confirm Lapicque in those very matters upon which his theory was based. Boehm excited muscles with condenser discharges of different rates and found that the constants of the Hoorweg equation altered when indirect excitation failed, but the new values were independent of the strength of curare or of the duration of its action. This work of Boehm therefore is consistent with the view that curare has no effect upon the excitation time of muscle or nerve but merely blocks the impulse at the myoneural junction. The observations are in complete contradiction with those upon which Lapicque built his theory.

Three years before Boehm published his failure to confirm Lapicque's experiments, however, some observations of Keith Lucas raised objections of a different kind to Lapicque's theory of curarization. Lucas [1907–8*a*] stimulated the sartorius muscle through large fluid electrodes and obtained strength-duration curves with kinks in them which he considered to indicate the presence of three excitable substances. The slowest (α) was found in the nerve-free part of the muscle, was unaltered by curare and was identified with muscle, the fastest (β) was found only in the neural part of the muscle but not in a nerve trunk, it persisted after a dose of curare sufficient to abolish indirect excitation, and

was therefore identified with some intermediate substance between muscle and nerve. The γ excitability had the same excitation time as nerve and was abolished by a strength of curare which just removed indirect excitability and hence was identified with nerve.

It is clear that Lucas's results are completely contrary to the expression of Lapicque's theory, for neither are muscle and nerve normally isochronous, nor does curare have any effect upon the excitation of muscle.

The reason for the great divergence in the results of Lucas and Lapicque was brought to light in 1923 by the experiments of Jinnaka and Azuma, who used the nerve-free portion of the sartorius and stimulated with both large fluid, and small pore electrodes. They found that the excitation time of the muscle depended in a great measure upon the size of electrode used, but restricted their curare investigations to observations with pore electrodes, which they considered to produce a smaller "deformation" of current. In their experiments they avoided confusion between muscle and intramuscular nerve fibres by the use of their micro-electrodes, but their work failed to establish the effect of curare upon muscle excitation time, since they make no statement as to whether the doses of 0·03 and 0·05 p.c. employed actually removed indirect excitation or not. It is clear that they supposed that the smaller dose was sufficient, but attributed [with Mines, 1908] the effect upon excitation time to the presence of inorganic salts in the drug. They certainly found that Ringer's fluid containing 0·046 p.c. KCl caused the excitation time of the muscle to become considerably longer than did the strongest dose of curare, but no information is given as to whether this Ringer curarized or not. These experiments therefore pointed the way to an accurate analysis, but failed to supply the requisite controls, and it is not until this year that the method has been brought to full fruition by the work of Grundfest (see later).

Long before this, however, Watts [1925] extended the methods of Jinnaka and Azuma to give a contribution so definite that it has deserved a recognition far wider than it has received.

He stimulated muscles with electrodes of various sizes and investigated normal muscles, those after nerve degeneration and after paralysis by curare of various strengths. The experiments were not such as to allow accurate measurements both before and after treatment without shift of electrodes, but it is perfectly clear that weak but paralysing doses of curare do not appreciably lengthen the muscle excitation time (in fact they shortened it) and degeneration (about 40 days) had also practically no effect upon it.

In view of this historical summary therefore it is seen that the views of Lapicque do not in the first place necessarily follow from his experiments, and the experimental results themselves are denied by the work of Boehm. Finally, the more penetrating analysis of Watts showed that normal isochronism was true or not, merely depending upon the size of electrodes employed (confirming Jinnaka and Azuma [1923] and Davis [1923]), but no matter what the size, there was no necessary change in the excitation time of muscle when paralysed by curare.

The case for Lapicque's theory which is most complete and best known to physiologists is the account in his book [1926]. The weight of this argument, however, is very largely offset when it is recollected that Lucas's contrary experiments were discredited on the supposition (since retracted) that they were due to certain errors, that Jinnaka and Azuma are only discussed where their experiments do not bear upon the question of curarization, and that no mention whatever is made of Boehm or of Watts.

In view of the foregoing considerations it was apparent that whatever the actual truth of Lapicque's views, the evidence put forward to support them needed considerable strengthening. In particular, experiments were needed which would distinguish clearly between the excitation of muscle and nerve in the fresh uncurarized condition, and which would give comparable results before and after treatment. Two methods presented themselves as the result of the observations of previous workers, either the use of large fluid electrodes in which case the muscle strength-duration curve is quite different from that of nerve, or the use of capillary pore electrodes where the stimulus may be localized to one particular excitable element. I adopted the first method and Grundfest the second, and it is satisfactory to find that in spite of the considerable difference in the techniques involved, our conclusions are identical.

The principle of the analysis by fluid electrodes is as follows. When muscles are stimulated in this way the strength-duration curve is often formed of two portions meeting at a kink as Lucas found [1907–8*a*]. If certain precautions are taken it can be shown that of these two portions the briefer (γ) is due to the intramuscular nerve twigs and the slower (α) to the normal muscle fibres themselves; I have never observed β. If this identification is accepted it is easy to investigate the effect of drugs, etc., upon these two curves and hence distinguish at once the action upon muscle from that upon nerve.

It was not possible, however, to assume at once the above identification of α and γ in view of the strong opposition of Lapicque. This

opposition I claim to have met in a series of six papers in this *Journal*, the results of which are summarized in the last of them [Rushton, 1932*c*], and will not be repeated here.

Curare and Block Electrodes[1].

Upon the floor of a rectangular trough [Rushton, 1930*b*, Fig. 6] filled with Ringer's fluid a frog's sartorius was fixed by a pin through the pelvis and a thread from the tibial tendon to a light tension lever. The current was led through non-polarizable electrodes of Zn-$ZnSO_4$-Agar-Ringer, and was localized to the pelvic portion of the muscle by being concentrated under a Bakelite block 1 cm. broad and of thickness to fit the section of the trough, and so placed that its pelvic edge lay about 2 mm. from the pelvic bone. In this position when the cathode lay towards the pelvis the strength-duration curve was entirely α, but on reversing the current the curve was largely γ with a small α tail appearing at long durations below the γ rheobase (see Fig. 2). By this means a full α or a full γ curve could be observed by merely reversing the current.

A resistance of 10,000 ohms was placed in series with the tissue, and durations of current were controlled either by a Lucas long-range pendulum or by condenser discharges; the current strengths were varied by a high resistance potentiometer used in conjunction with an accurate voltmeter.

It is a familiar fact that the strength-duration curve of an excised sartorius changes considerably in the first few hours after excision, probably due to attaining a new equilibrium with the bathing fluid. In order to avoid complications due to these changes, muscles were not curarized until they had been left in Ringer's fluid for an hour or two and when the strength-duration curves appeared to have reached a stable condition. When at last equilibrium had been attained it was important not to disturb the salt balance in the addition of curare solution. To this end the curare (Merk) was made up concentrated in Ringer's fluid (1·0 p.c.) and 0·1 c.c. of this solution added to the 10 c.c. of fluid in equilibrium with the muscle. It was found that, after mixing, this concentration of the drug abolished excitation through the nerve in 10 min. In all these experiments the nerve to the sartorius was dissected out and placed (well away from the principal electrodes) on a subsidiary pair through which induction shocks could be passed. The moment of curarization could thus be obtained with certainty.

[1] These experiments were communicated to the American Physiological Society in March, 1930 [Rushton, 1930*a*].

Results.

The results of a typical experiment are shown in Fig. 1 plotted in logarithmic scales on both axes. The advantage of this manner of plotting (which has been discussed at the end of a former paper [Rushton, 1931]) is in the present case that the curve never runs too steeply to be easily followed, that the limits of error and exact positions of the experimental points may be clearly seen, that there is no confusion between the several curves appearing on a single figure, and that change of rheobase without

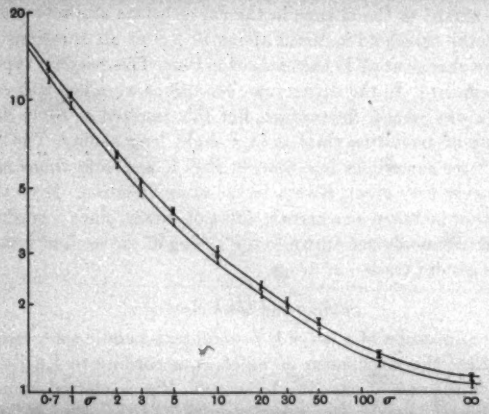

Fig. 1. Strength-duration curve of sartorius stimulated at pelvic end through block electrodes. Lower curve before curarization, upper curve after curarization and half an hour later. Abscissae log duration in σ, ordinates log strength of current.

change of excitation time is at once appreciated since in this case the whole curve moves bodily upwards or downwards vertically.

The procedure for the experiment of Fig. 1 was as follows:

The sartorius muscle with its nerve was dissected out in the morning and set up in the stimulating trough placed in a thermally insulated box cooled with ice to a stable temperature of 13·5° C.

3.30 p.m. The strength-duration curve was taken without opening the box (lower curve, Fig. 1).
4.01 p.m. The box was opened for a moment to administer a drop of concentrated curare, to make a solution of 0·02 p.c.
4.07 p.m. Slight response still from shock to nerve.
4.08 p.m. Curarization complete.

4.09–4.19 p.m. Strength-duration curve redetermined (upper curve right point of pair, Fig. 1).
4.40 p.m. Strength-duration curve repeated (upper curve left point of pair, Fig. 1).
Final temperature 12·5° C.

From the figure it is clear that the continued action of curare between 4.10 and 4.40 had not the slightest effect upon the threshold at any duration. It is interesting to note, however, that during this interval, a gastrocnemius muscle (from the same frog) which had also been placed in the trough, was gradually being curarized. This was accomplished at 4.39 p.m., taking 5½ times as long as the sartorius.

With regard to the change in the curve before and after curare it is seen that the threshold is raised about 10 p.c. at all durations and that there is no change at all in the excitation time. This result is typical of all my experiments. In the earlier ones conditions were less well controlled and there was greater fluctuation, but this resulted as often in a slight shortening of excitation time as in a slight lengthening. The threshold changes were sometimes less than in Fig. 1, and sometimes more, and though never very great, always in the same direction. Even this, however, cannot be taken as a certain effect of curare, since a gradual rise of threshold commonly accompanies the ageing of the excised muscle apart from the administration of drugs.

Fatigue and block electrodes.

The tetanization of a nerve to a voluntary muscle soon brings about curarization, the mechanism of which is, according to Lapicque, the same as in the case of paralysis by curare. Fig. 2, plotted upon uniform scales, gives the result of an experiment to find the effect of fatigue and subsequent curarization upon the α and γ curves of muscle.

The sartorius was set up as before and strength-duration curves were obtained (crosses) with the current towards the tibia (intermittent curve) and towards the pelvis (full curve). The nerve was then tetanized to complete failure of indirect excitation. The threshold was found to be the same whether the tetanizing current continued to be applied throughout, or whether it was stopped for a second while each strength-duration stimulus was sent in. It is seen (white circles) that curarization by fatigue has no effect at all upon the α curve obtained from the pelvic end of the muscle. On the other hand, the γ curve, initially prominent where the cathode lay towards the tibia, has completely vanished and in its place is seen an α curve which exactly joins the α portion initially appearing below the γ rheobase at durations greater than 15σ.

It seemed of interest to observe the effect of curare upon the muscle

tetanized as above. According to the action claimed by Lapicque it might be supposed that the excitation time of muscle would be increased by the drug, since he expressly states [1925] that there is no fixed upper limit to the muscle excitation time which might otherwise already have been attained by tetanization.

The effect of curare in these circumstances is seen in Fig. 2 (black circles), where it will be observed that there is no change whatsoever in the curve with cathode towards pelvis. With the reversed direction of the

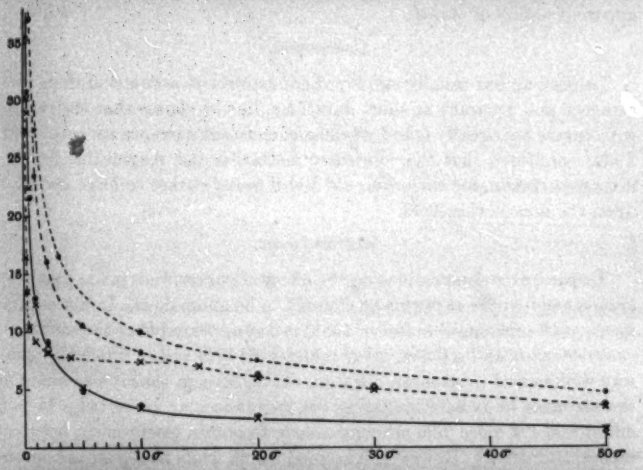

Fig. 2. Strength-duration curve of sartorius stimulated through block electrodes. Full curve cathode towards pelvis, intermittent curves, anode towards pelvis. Crosses before fatigue, white circles after fatigue, black circles after curare. Abscissae duration in σ, ordinates current strength.

current there is a small rise of threshold but no change in excitation time, hence not even in the region of nerve endings is any change in time relations observable.

Unfortunately this gives no information as to whether curare has any effect on the excitability of a muscle in the region of the nerve ending. If the drug made the muscle locally more irritable this would have appeared as a lowering of threshold, but if the muscle suffered the reverse change the only observed result would be the possible shifting of the most excitable fibre to one whose nerve did not enter in the neighbourhood of the applied cathode. This would be manifested as a slight rise in threshold for descending currents with

no change in threshold for ascending currents—which is in fact what is observed, but the evidence is far too slender to infer that the results were necessarily produced in the above manner.

These experiments therefore confirm the results of Lucas and Watts with large electrodes, for not only is there no change of excitation time due to curarization by fatigue or curare, but also there is practically no threshold change at all.

I next sought to discover which of the differences between Lapicque's technique and my own were responsible for the differences in the apparent action of curare.

Condensers.

Lapicque has usually employed condensers on account of their convenience and accuracy at short durations, but he claims that the results with curare are equally found when brief constant currents are employed. I also confirmed that the condenser method is not responsible for the divergent results, for with their aid I still found curare to have no effect upon the muscle threshold.

Gastrocnemius.

Lapicque's observations on the effect of curare were made upon the gastrocnemius: the sartorius he claimed to be anomalous. In my experiments with undrugged muscles [1931] it had appeared that the sartorius, gastrocnemius and a dozen other muscles all behaved in much the same way with regard to strength-duration curves, except that it was easier in the sartorius to avoid stimulating the intramuscular nerve twigs (which might make it more difficult consistently to obtain isochronism between muscle and nerve). However, it seemed worth while to repeat the curare experiments upon the gastrocnemius, using fluid electrodes and the same technique as before. The result of one experiment is shown in Fig. 3 (plotted in logarithmic scales), where the procedure was as follows:

Temp. 12° C.	4.0 p.m.	Normal strength-duration curve (intermittent line).
	4.15 p.m.	30 mg. curare added to bathing fluid (10 c.c.).
	4.47 p.m.	Curarization complete: strength-duration curve (crosses).
	5.48 p.m.	Strength-duration curve (white circles).
	9.48 p.m.	Strength-duration curve (black circles).
Temp. 12° C.		

It is seen at once that the results here are the same as with the sartorius. The initial curve shows a prominent γ portion because of the difficulty of finding a nerve-free part of this muscle, but there is quite

sufficient of the muscle curve (durations greater than 7σ) to make it clear that after curarization (crosses) the threshold and excitation time are unaltered. As for the continued action of the drug prolonging more and more the muscle excitation time, so far from this being the case, after 5 hours' immersion there is no change that cannot be put down to alteration of ionic equilibrium (much slower than with the sartorius), and in any case the excitation time is not prolonged, but diminished.

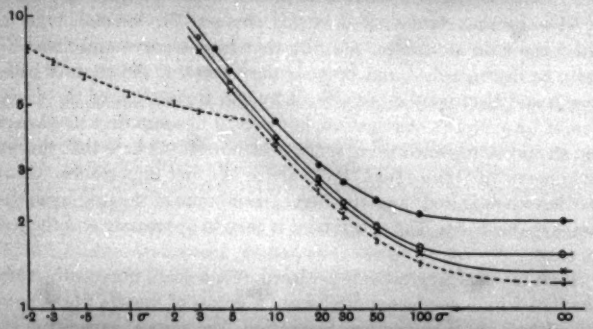

Fig. 3. Strength-duration curve of gastrocnemius stimulated through block electrodes. Intermittent curve before curarization, crosses, white circles, black circles, immediately after curarization, 1 hour, and 5 hours after curarization respectively. Abscissae log duration in σ, ordinates log strength of current.

Nature of the curare.

The curare which is placed upon the market is by no means a pure chemical, and it seemed quite possible that among the constituents there were two quite distinct substances one of which paralysed and the other independently altered the excitation time of muscle. According to this idea the Sorbonne curare had a much larger quantity of the second constituent than either the Cambridge curare, the sample which I obtained from Merck, or the specially purified product used by Boehm. I was enabled to investigate this possibility through the kindness of Prof. Lapicque, who sent me two samples of the drug one of which was from the supply which he had used for most of his experiments (curare "Type").

This curare was very effective in removing indirect excitation, but otherwise behaved exactly like the sample with which I had already experimented. In fact the actual examples quoted in Figs. 1 and 3 were

performed with Lapicque's "curare type." It is clear, therefore, that whatever differences may lie in different samples of curare, the increased excitation time of muscle observed by Lapicque cannot be attributed to this cause. This has been further confirmed by Lapicque, who finds [1930] that with large electrodes the excitation time is if anything diminished by curare but with stigmatic electrodes it is greatly prolonged.

Nature of electrodes.

The experiments described in this paper so far have all been made with large fluid electrodes, since by that means nerve and muscle may easily be distinguished and because the nature of the contact between muscle and electrodes is not altered by the application of the drug. In view of Lapicque's observation, however, it appears that the investigation should be repeated using small electrodes. It is here that the recent experiments of Grundfest [1932] are of the first importance. Grundfest has investigated the retro-lingual membrane of the frog by means of capillary electrodes. The experiment is easy to appreciate and the results are clear.

First the capillary cathode is placed over a small nerve twig which is stimulated by a condenser discharge. A group of muscle fibres respond and of these one is chosen for observation. The voltage-capacity curve of the nerve is then taken using this muscle fibre as index. Next the cathode is applied directly to this muscle fibre and the voltage-capacity curve obtained, when it is thus directly excited. The cathode is replaced on the nerve, curare is added, and the efficacy of curarization ascertained by failure of response to indirect stimulation. Finally the cathode is again placed on the muscle as before and the voltage-capacity curve redetermined.

The results are quite definite. Initially the excitation time for nerve is about ten times as brief as for muscle. Curare has no effect whatever upon the excitability of muscle, either in threshold or excitation time. These results are therefore in complete accord with those where large electrodes are used and directly contradict Lapicque's claim as to the difference in the two cases.

In order to complete this type of investigation it seemed worth while to repeat these observations with electrodes larger than Grundfest's and more akin to those of Lapicque. First I attempted to use an Ag-AgCl wire applied vertically to the surface of the pelvic region of the sartorius but not pressing. I found it impossible to get reproducible results by this method even in the absence of drugs, and this at the time

I attributed to the polarizability of the stigmatic electrode (even though the stimulus was reversed between each observation). A more probable explanation, however, is the toxic effect of this form of electrode when applied directly to muscle [Rushton, 1932*b*]. More repeatable results were obtained by using a relatively large capillary, of internal diameter 0·26 mm., applied to the pelvic end of the sartorius, the large indifferent anode being placed over the point of nerve entry. Whether the muscle was covered with Ringer or whether it was in air when strength-duration curves were taken, made no difference to the result that curare in a dose sufficient to abolish indirect excitation had no effect upon the muscle excitation time.

The following two examples are typical.

Exp. 1. Preparation set up at 12.0 m. as described, with 12,000 ohms equivalent series resistance in condenser circuit.

12.0 m. *Rheobase* 7·5 volts. *Excitation time* 0·046–0·044μF.
2.0 p.m. *Rheobase* 15·0 volts. *Excitation time* 0·084–0·080μF.
Langley's curare added, abolishing the indirect excitability in 3 min. The excitation time was now 0·086–0·083μF.

Exp. 2. Preparation set up at 10.5 a.m., resistance in condenser circuit 10,000 ohms.
10.40 a.m. *Rheobase*, 4·0–3·8 volts. *Excitation time*, 0·084–0·080μF.
10.55 a.m. Langley's curare added, abolishing indirect excitability between 11.0 and 11.1 a.m.
11.5 a.m. *Rheobase*, 4·0–3·8 volts. *Excitation time*, 0·094–0·090μF.
11.45 a.m. *Rheobase*, 5·5–5·2 volts. *Excitation time*, 0·090–0·086μF.

From Exp. 1 it is seen that there is a large change in the excitation time of the excised muscle in the first hour or so after excision and setting up in Ringer's fluid due to the new ionic balance. This change is nearly in the ratio 1:2, yet no sign of conduction block was ever seen on indirect excitation. Obviously if curare were administered in this interval the impression might arise that it was the drug which was prolonging the excitation time. This is clearly incorrect, for not only does this prolongation occur without the application of curare, but also the subsequent administration of the drug produces no change in excitation time at all. This is confirmed by Exp. 2, where it is seen moreover that the prolonged action of the drug produces no effect. These experiments clearly exhibit a fair degree of reliability and stability of threshold, and the results are in complete accord with Grundfest and the earlier conclusions of this paper.

Strength of curare.

In all my experiments a weak solution of curare was used, 0·01–0·02 p.c., which curarizes the sartorius in 5–10 min. but takes nearly an hour with the gastrocnemius. It is certain that in some, and possibly in most

of Lapicque's experiments a much stronger solution was used, and it is easily conceivable that such a solution might prolong the muscle excitation time. I have not investigated this point, since it is clear that if a weak but curarizing dose does not alter excitability, the chronological theory falls, no matter what results arise with stronger doses. Moreover, since Mines showed [1908] that the excitability change produced by the drug was the same as that produced by the ash of an equal quantity of drug (hence due to its inorganic content) it is not unlikely that this matter is merely one of salt balance and has nothing to do with curarization.

We may summarize the analysis of Lapicque's first argument as follows:

(i) In all the experiments known to me where the direct excitation of muscle has been clearly distinguished from the excitation of intramuscular nerve twigs, nerves have always been found to have a shorter excitation time than muscle measured with the same electrodes whatever the size.

(ii) Curare has no action upon the excitation time of muscle as observed by Boehm or myself using the same size of electrodes as Lapicque, or by Grundfest using smaller ones, or by Lucas, Watts or myself using larger ones.

Thus with regard to Lapicque's first argument to support his theory of curarization, we have seen that in the first place it is inconclusive, admitting equally of the conclusion of initial heterochronism; in the second place, even granting his observations, there is no reason to suppose that the change of excitation time which he obtains is in any way related to the curarizing action of drugs; and in the third place his observations are denied by all investigators whose work has clearly distinguished between the direct excitation of muscle and the stimulation of nerve twigs.

We pass to Lapicque's second argument.

Lapicque's second argument.

The second of the three arguments employed by Lapicque to justify his chronological theory of excitation relates to an antagonism which he finds between the drugs strychnine and veratrine with regard to their curarizing actions. The argument is briefly as follows. Strychnine has no effect upon the excitation time of muscle, but that of nerve is shortened and at the moment of abolition of indirect excitability there is a heterochronism in the ratio 1:2. Curarization is supposed to be the result of this heterochronism as in the case of curare. Veratrine on the other hand

curarizes at a moment when the nerve excitation time is unaltered, but that of the muscle shortened. Now if in fact curarization is due to divergence in excitation time, it is clear that after curarization by veratrine indirect excitation should be restored by strychnine or *vice versa*. This in fact is claimed to be the case, thereby constituting an interesting confirmation of the theory.

But evidence which is amply adequate for the confirmation of a theory may be quite insufficient for its initial establishment and it is very doubtful whether the strychnine-veratrine antagonism would ever have led to Lapicque's theory if he had not already put it forward upon other grounds. Those grounds we have already considered in the previous section of the present paper and have seen that they are very inadequate, for not only is the theory not supported, but it is definitely rendered untenable by the observation that paralysis by curare may (and in my experience always does) occur without any change in the excitation time of muscle or nerve. The most favourable observations of strychnine-veratrine antagonism, therefore, could not justify Lapicque's theory in the face of the foregoing evidence. Yet since a systematic correlation between drug antagonism and the opposite effects of the individual drugs upon the excitation time of nerve and muscle might not be without value, I have attempted to confirm Lapicque's observations upon this matter. It will be unnecessary, however, to describe the majority of the various experiments performed, since that which is at once the simplest and most crucial shows that strychnine and veratrine do *not* antagonize each other.

In this experiment I followed Lapicque in that I used the frog's sciatic gastrocnemius preparation, and employed the solutions he mentions [1926] namely strychnine HCl 0·3 p.c., veratrine HCl 0·1 p.c. In the first variety of this experiment the central end of the nerve was screened from the drug and electrodes were applied to the screened portion. By this arrangement the excitability of the nerve under the electrodes was unaffected by drugs, and the possibility of a conduction block occurring at the place where the nerve passed from the screened to the unscreened region was excluded by controls which showed that conduction block was never produced in this way. By means of a simple piece of mechanism an induction shock was applied to the nerve automatically every minute, and the resulting contraction recorded on a very slowly moving drum. The results of two typical experiments are shown in Fig. 4, where the tracing is to be read from right to left. Owing to an imperfection in the mechanical contact, occasionally a double shock was administered pro-

ducing summation, but apart from this, the records are very regular. In the upper tracing there is a uniform height when in Ringer's fluid and immediate summation and contracture when 0·1 p.c. veratrine was added followed by a rapid decline with complete curarization in half an hour. Now the veratrine was removed and replaced by 0·3 p.c. strychnine, but though the record was continued for several hours beyond what is shown in the figure, no sign of a twitch was observed. These strengths of drug were as recommended by Lapicque, and yet the strychnine seems still too weak to neutralize the veratrine. The lower tracing therefore shows the repetition of the experiment on the other leg of the same frog where

Fig. 4. The effect of veratrine and subsequent application of strychnine upon nerve-muscle conduction (see text).

the strychnine-veratrine ratio was increased nine times. As would be expected paralysis is slower, but here too there is no restitution of conduction. There was no single exception to this result; never for the space of a minute did strychnine restore the conduction abolished by veratrine, although the strychnine solution was so strong that one drop in the lymph sac of a spinal frog produced an intense spasm within 5 min.

Prof. A. V. Hill was good enough to point out an important possibility which must be considered, however, in connection with this analysis. It is known that in a veratrine contracture a very great deal of energy is expended, hence in the repeated contractures which were elicited in the foregoing experiment a considerable degree of fatigue was

doubtless attained. The question arises as to whether curarization was due to fatigue and not directly to veratrine at all, for if this were the case no resuscitation by strychnine would be expected.

To investigate this possibility the experiment was modified slightly as follows. Two preparations, *A* and *B*, from the same frog were set up side by side in the way described above, and after initially ascertaining that both were irritable and in good condition shocks were applied to *A* only. It is obvious that if curarization of *A* is due to fatigue as suggested, *B* will always be excitable at the moment when *A* fails. If on the other hand the fatigue has nothing to do with the question *B* will be paralysed before *A* as often as not. This latter was actually the case, for in slightly more than half the experiments *B* had already been curarized by the time that *A* failed. A typical example is as follows:

4.20 p.m. 0·1 p.c. veratrine HCl solution added.
Every 2 min. a shock is sent into preparation *A*; *B* is rested.
4.44 p.m. *A* still contracts.
4.46 p.m. *A* curarized; *B* curarized, though now excited for first time.
4.48 p.m. Veratrine poured away and 0·3 p.c. strychnine HCl added.
4.50–5.50 p.m. Shocks applied to both preparations every minute for next hour without ever evoking a response.

From results such as those it is clear that curarization of *B* cannot be due to fatigue, since there was no fatigue and hence in these cases at least Lapicque's drug neutralization does not occur. Since, moreover, the evidence leads us to suppose that even when fatigue is present it has no effect on the veratrine curarization, we may also accept the experiment of Fig. 4 as trustworthy.

But though the foregoing experiments have shown that strychnine will not antagonize the veratrine curarization when used in Lapicque's concentrations or when the strychnine-veratrine ratio is considerably increased, are we to conclude that Lapicque's theory is inoperative? Is not the alternative equally plausible, that the strychnine used was at strongest too weak to reduce the nerve excitation time to the diminished value which the applied veratrine has produced in the muscle? A slight modification of the foregoing experiments gives definite evidence on this matter. It is obvious that if strychnine neutralizes the action of veratrine as Lapicque supposes, by shortening the excitation time of the nerve to the diminished value assumed by the drugged muscle, then, equally, veratrine will neutralize the curarization with strychnine by bringing about the same final condition. This order of performing the experiment avoids completely the complications of the veratrine contracture, since

veratrine is not applied till after curarization by strychnine has been brought about, and in no case was any further movement of the muscle observed.

A typical experiment is as follows:

5.5 p.m.	Preparation immersed in 0·3 p.c. strychnine HCl.
5.30 p.m.	Still excitable.
5.35 p.m.	Curarized.
5.37 p.m.	Strychnine replaced by 0·1 p.c. veratrine HCl.

The preparation was stimulated for the next half hour at first every ½ min., later at 1 min. and 2 min. intervals, but no excitation ever returned.

It is seen that this case confirms all the foregoing. Curarization by strychnine could never be abolished by the replacement of this drug by veratrine in the strengths used by Lapicque. These results, moreover, cannot be explained upon the grounds of unsuitable drug concentrations, since in this case the *veratrine* would have to be too weak to reduce the muscle-excitation time to the diminished value produced in the nerve by the strychnine, which is precisely contrary to the conclusion of the previous experiment. In short, there is never restoration of conduction, no matter which drug is applied first, and hence this failure cannot be due to the first drug being always the stronger.

In all these experiments there can be no question of large or small electrodes, true or false chronaxies. We have here only to consider whether an impulse can or cannot be conducted, whether a muscle twitches or not. If Lapicque's theory were true, the antagonism would have to occur every time—a few exceptions would render it untenable. But in my experience the exceptions so far from being few are universal. In no single case have I ever seen this antagonism which Lapicque's theory would inevitably demand.

With regard to Lapicque's second argument therefore, we may conclude that at best the strychnine-veratrine antagonism could only confirm by exceptionless regularity a theory established upon other grounds. But these other grounds are wanting, and the drug antagonism is in my experience never obtained.

We pass to Lapicque's third argument.

Lapicque's third argument.

Lapicque's third argument in favour of his chronological theory of curarization is comparatively recent [1925] and makes use of the fact investigated by von Kries [1884] and Keith Lucas [1907–8*b*] and

himself [1908] that if a current increases from zero somewhat slowly it may never excite a nerve but may still be capable of eliciting a propagated contraction from muscle. As a means of demonstrating the presence of muscle in the nerve-muscle complex this method is a very elegant one, but it gives us no information about the excitation time of the muscle unless we assume with Lapicque that the minimum rate of current increase which will excite is inversely proportional to the excitation time, whatever the tissue and whatever the condition of the tissue. This is a very bold assumption, and some substantiation would surely not have been out of place, especially since the only experiments I know which test the matter directly [Lucas, 1908] show quite contrary results. The only justification which is given, however, is the following mathematical generalization. If the stimulating current is any function of time $f(t)$, and if τ is the excitation time of the tissue then $f\left(\frac{t}{\tau}\right)$ is an expression independent of the tissue investigated or of the condition of the tissue. This generalization is far more sweeping than the particular case of it considered by Lapicque in his third argument, and consequently it needs the more extended substantiation. The proof of such a statement must be a most laborious work, involving as it does stimuli of diverse kinds, applied to various tissues, in all sorts of conditions. Whether Lapicque's generalization has in fact ever been supported by such a series of investigations or not, I do not know. I am only aware of one particular case which has been studied with the necessary care—that of the strength-duration curve—and here $f\left(\frac{t}{\tau}\right)$ is not independent of the tissue, being what Lapicque calls "canonical" in slowly reacting tissues but "uncanonical" in frogs' nerves [Rushton, 1932*a*].

As a result of this analytical survey therefore we may conclude that Lapicque's generalization is at best unproven, and probably incorrect, and that the particular case of it which he uses for his third argument lies in the same category. Thus even if we accept his experimental conclusions we cannot deduce anything from them concerning the excitation time of the tissues involved nor learn anything concerning his chronological theory of excitation. The conclusions in question, however, were not experimentally confirmed.

Lapicque's experiment [1925] was as follows. The nerve-muscle complex is excited directly through a stigmatic cathode by the apparatus shown in Fig. 5. If first the capacity C is made zero, then when the key K is closed a constant current will flow through the muscle, as shown in Fig. 6 *A*. The threshold is determined, and is in fact the rheobase. Now a

small capacity is given to C, and when K is closed the current through the tissue increases as shown in Fig. 6 B. The threshold for this current will be greater than the rheobase. If now the capacity of C is still further increased the current will rise still more slowly on closing K (Fig. 6 C) and the threshold will be still higher.

The consideration of the curve relating the threshold with the capacity of C both before and after curare led Lapicque to two conclusions:

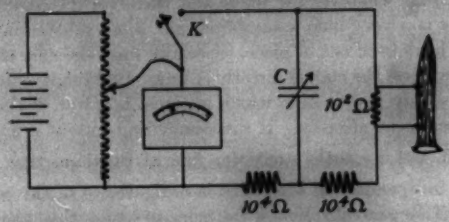

Fig. 5. Lapicque's circuit for excitation with slowly increasing currents.

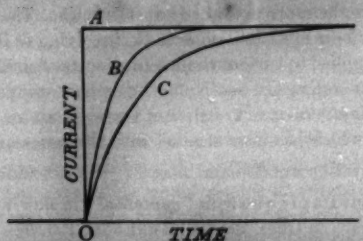

Fig. 6. Time course of current through muscle on closing K, Fig. 5.

(*a*) Since before curare the curve was the same as for nerve he concludes that nerve and muscle are normally isochronous.

(*b*) Since after curare the threshold for abrupt currents is raised while for slowly rising currents it is lowered he concludes that curare prolongs the excitation time of muscle.

But even if we neglect the foregoing criticism neither of these conclusions can be accepted without further investigation. For (*a*) may be equally interpreted as due to the intramuscular nerves being excited, in which case naturally the curve would be the same as in the case of the nerve trunk, and we learn nothing about the muscle except that its threshold is higher than that of nerve—the rheobase perhaps three times

as great. As Lapicque mentions no precautions to exclude nerve twigs this must be regarded as a very likely explanation. But in this case (*b*) may merely signify that after curare has removed the nerves, the remaining curve is the normal muscle curve, unaffected by the drug. Against this Lapicque argues that were it the case the lower threshold at large capacities would have appeared in the initial curve (the lowest threshold in every case obviously being taken). The validity of this depends upon the stability of the threshold measurements. Clearly if during the process of curarization there was a change in the exact point of stimulation, in the degree of moisture of the surface, or of the nature of the contact, etc., these physical conditions might produce considerable threshold changes. Lapicque himself, in urging the relative stability of the chronaxie as a measure, has emphasized the variability of the rheobase—at least when obtained through stigmatic electrodes in air. Can we rely upon this rheobase before and after curarization to be so stable that some lowering due to physical causes is out of the question? It seemed worth while to repeat Lapicque's experiments bearing in mind these criticisms.

To this end I followed Lapicque's methods as closely as was compatible with certain controls. The electric circuit was exactly as described [Lapicque, 1925]. It was found impossible to get repeatable results using Ag-AgCl electrodes or with muscle completely in air, hence a glass capillary of 0·26 mm. bore was used as cathode and applied to the surface of the muscle just emerging from a Ringer bath. The muscle used was the frog's sartorius with nerve dissected out; the cathode was placed near the pelvic extremity; the anode, which was a tube of 7 mm. diameter, was fixed over the place of nerve entry. From a former paper [Rushton, 1932*b*] it is clear that nerves are less likely to be excited in the nerve-muscle complex if the anode is placed over the nerve entry than in any other position.

By tilting the trough it was possible to immerse the muscle or cause the fluid to drain back again without disturbing the electrodes, and if a minute or so was allowed for drainage the results were found to be very fairly reproducible as the following example shows. The threshold for each case lies between the two figures quoted; their difference shows the experimental limits.

The preparation was set up at 4.10 p.m., and it is clear that over the first hour there is a considerable variation due to change of equilibrium. Obviously anyone curarizing in this period might be led to suppose that it was the curare which was raising thresholds for small capacities more than for large (as Lapicque claims). It is moreover interesting to note

that although the change in the first two columns are almost as great as those which Lapicque finds with curare, yet no sign of curarization was revealed by exciting through the nerve. On the other hand, adding a drop of concentrated curare to the bathing fluid and tilting the trough caused curarization in 8·5–9 min., without producing any change other than a slight rise of threshold for all capacities. The figures show beyond doubt that neither at the moment of curarization nor for 40 min. afterwards was there any significant change of threshold due to curare, which only confirms the earlier experiments of this paper. This experiment, which is typical of all which I have performed, therefore, leads to the following conclusions.

Thresholds.

	Before curarization		After curarization		
Capacity	4.30 p.m.	5.0 p.m.	5.20 p.m.	5.25 p.m.	6.0 p.m.
0·0$_{\mu}$F	9·0– 8·5	13·5–13·0	14·0–13·5	14·5–14·0	13·5–13·0
0·3$_{\mu}$F	9·5– 9·0	13·5–13·0	14·5–14·0	15·0–14·5	14·0–13·5
1·0$_{\mu}$F	11·5–11·0	14·0–13·5	15·0–14·5	15·5–15·0	14·5–14·0
3·0$_{\mu}$F	14·5–14·0	15·5–15·0	17·0–16·5	17·0–16·5	16·5–16·0
10·0$_{\mu}$F	22·0–21·0	20·0–19·0	22·0–21·0	23·0–22·0	22·0–21·0
0·0$_{\mu}$F	9·0– 8·5	13·3–12·8	14·5–14·0	14·0–13·5	14·0–13·5

Curare added at 5.10 p.m., indirect excitation abolished between 5.18½ and 5.19 p.m.

When first set up the muscle is unstable and no observations are significant for an hour or so (confirming former investigators). When the capacity-threshold relation is taken this does not coincide with Lapicque's results with nerve, but more closely with his results with muscle after nerves had been removed by curare. All thresholds after curare are (within experimental limits) exactly the same as before curare.

These results are quite irreconcilable with Lapicque's conclusions, but they are easily reconcilable with his observations which apparently should be interpreted as suggested earlier, namely, that initially he was stimulating nerve twigs, and when these had been removed by curare he was stimulating the normal muscle.

To summarize. Lapicque's third argument deals with the excitability of tissues to slowly rising currents. No relation was established, however, between this form of excitation and the excitation time, hence measurements upon the one could not be used to establish a curarization theory involving the other, however sound the experimental conclusions. These conclusions, however, lay themselves open to an entirely different interpretation, which the present experiments confirm, namely that curare has no effect upon the excitability of muscle.

DISCUSSION.

In the former parts of this paper we have considered the evidence which Lapicque has brought forward in support of his chronological theory, and have observed that each of his arguments is doubly inconclusive. In the first place the deductions which are drawn from the experiments are not inevitable, and explanations other than Lapicque's would fit equally well—on this account the chronological theory is possible but unproven. In the second place the very experiments are not confirmed when various precautions are taken as already described—on this account the chronological theory is not merely unsupported but untenable.

Whatever may be the mode of action of curare, it certainly is not to alter the excitation time of the muscle fibre at least over the greater part of this structure. Whatever may cause conduction block it is not the production of heterochronism, for no observable change in excitability exists.

If then chronaxie does not play the important physiological rôle that has been attributed to it, if it is not the pass-sign of nerve-muscle conduction, what, finally is the value of this measure? This greatly depends upon the meaning we assign to "chronaxie." If we mean a measure satisfying all Lapicque's recent restrictions—obtained only from a curve which is canonical, with electrodes not large enough to give the "α complication" nor small enough to give the "Grundfest heterochronism"—then clearly the utility of chronaxie will be limited. If a more general meaning is assigned, the measure has still great value in physiology, but to avoid confusion it is essential to use a name distinct from "Chronaxie," which according to Lapicque may no longer be employed in this sense. Lucas's "Excitation time" exactly meets our requirements. Excitation time therefore is the measure characterizing the rate of development of the excitatory process in given conditions.

It is important to note that in general the term "conditions" will comprise two factors, the tissue (involving its nature, temperature, salt balance, etc.) and the physical conditions of stimulation (involving the distribution of current over the surface of the tissue). In certain particular cases the second factor will make little difference to the result; for instance, a sciatic nerve gives an excitation time relatively independent of the nature of the electrodes, possibly because the nodes of Ranvier are the effective electrodes in this case and because all the nodes are very similar. But familiarity with nerves has given rise to the erroneous im-

pression that independence of the conditions of stimulation is the rule instead of the exception (even though with nerves there is appreciable dependence), and hence it must be emphasized that unless the contrary has been established it should always be assumed that excitation time will vary with the nature of the electrodes.

The reason for this variation has not yet been established with certainty, but since it follows as a logical consequence from the commonly accepted theory of excitation [Rushton, 1932*c*] this explanation seems obvious enough and might have been accepted without further comment had not Lapicque put forward an alternative [1932]. A theoretical discussion of the excitation process obviously lies outside the scope of the present paper, but since Lapicque's alternative assumes that excitation time in general does not measure the duration of the process of excitation but the sum of this and another large extraneous factor, it is important to consider this question when dealing with the measurement and significance of the excitation time.

It is generally agreed that the passage of the stimulating current through the tissue polarizes the membranes through which it passes and thus produces a counter E.M.F. which tends to disperse the polarization. For this reason the polarization is developed not only under the electrodes but gradually further and further away as has long been known from investigations upon electrotonus. Now from Nernst onwards it has been commonly supposed that excitation is the direct result of the establishment of a certain critical ionic concentration (at least if this be attained within a very brief interval), and so it is important in considering Lapicque's suggestion to know whether he accepts this view or not. In the absence of any explicit statement or discussion upon this head we should hardly have expected a new but tacit departure, yet from his paper not only is the above polarization treated as a phenomenon distinct from the basis of excitation, but apparently excitation itself cannot begin to develop until the polarization has been arrested. This polarization moreover has some surprising properties. When it spreads down the length of a fibre, for example, it is stopped by the wall of a glass tube placed outside the connective tissue over the surface of the muscle, and this stoppage apparently arrests the increasing polarization at a distant spot under the cathode.

However, apart from this doubtful physics Lapicque and I are in complete agreement in considering that excitation time measures the duration of the polarization process (at least when large fluid electrodes are concerned) and thus it measures that process which is generally

accepted as underlying excitation. The only divergence is that for some reason unspecified Lapicque appears to have tacitly rejected the classical connection between polarization and excitation, treating the two as separate and somewhat opposing entities, but explaining his position no further. There is obviously here no necessity for abandoning the polarization theory of excitation, and since there is general agreement that excitation time characterizes the rate of polarization, it consequently measures the rate of development of the excitatory process.

The arguments urged on theoretical grounds against large fluid electrodes fall with the theory upon which they were based, and electrodes of the block type not only are admissible but are probably the most satisfactory of any for work on the excitability of excised tissues. Among their advantages are the following:

(*a*) Stability of threshold resulting from the uniformity of ionic environment, the absence of desiccation, the constancy of contact, and the relative unimportance of small movements.

(*b*) Ease of investigating with stable threshold the effect of drugs, etc.

(*c*) Obvious distinction between excitation of muscles and nerve twigs (α and γ curves).

(*d*) Knowledge of the potential applied to every part of the surface of the tissue. This knowledge is a first essential for any theoretical treatment concerning the current entering the tissue, for without it the proportion of the applied current which enters cannot be known.

Conclusions.

Two and a half decades have passed since Lapicque first put forward his chronological theory of curarization, and during this time a very large number of publications have appeared based upon these ideas which have become widely accepted.

His position was definitely established when in 1926 he published his well-known book which by its exceptional interest and lucidity has deservedly won a place in every physiological library. For the main thesis of the book we can have nothing but admiration. It represents Lapicque's chief contribution to physiology, namely the demonstration that tissues of apparently the most diverse kinds differ essentially in only one respect—the scale of time in which they act. Extend or diminish this scale and the gross differences vanish. But the exposition of his theory of curarization could not be so unreservedly accepted. A chapter

of 40 pages deals in detail with this theory, and yet, as we have seen, the evidence is inconclusive, the experimental discussion has some important omissions, and the treatment of Lucas's results is not convincing. It was obvious that further work was still needed either definitely to establish or definitely to disprove this important theory which was yearly being more widely accepted as the basis of new departures.

During the past four years I have attempted to do this, and the present paper completes my investigation. The principal results which have emerged are the following:

(*a*) When any striated muscle is excited through fluid electrodes two kinds of strength-duration curves may be found either single or combined in proportions which may be altered at will. One of these (γ) is identical with nerve, the other (α) very much slower.

(*b*) A variety of experiments shows that α is due to the excitability of one element and γ of another.

(*c*) Various kinds of abnormalities were excluded, hence this dual excitability must be considered normal.

(*d*) A detailed analysis proves α to be muscle, and γ invariably the intramuscular nerve twigs.

(*e*) By this technique curare is found to have no action upon muscle excitability, for the α curve is unchanged, though the γ vanishes.

(*f*) Curare is also without effect upon the muscle strength-duration curve elicited by a capillary cathode applied to the nerve-free portion of the sartorius.

(*g*) Curare is also without effect upon the excitability of the muscle to slowly rising currents.

(*h*) Curarization by strychnine was never abolished by veratrine, nor *vice versa*.

In fact in these experiments where the direct excitation of muscle has been clearly distinguished from the excitation of the intramuscular nerve twigs, the latter have invariably been found to have a shorter excitation time than muscles stimulated through the same electrodes whatever their nature.

Neither curare nor fatigue has been found to have any effect whatever upon the muscle threshold no matter what the nature or duration of the stimulus.

These observations, therefore, do not substantiate Lapicque's theory of curarization, but on the contrary they render that theory untenable.

SUMMARY.

1. The paper attempts a critical and experimental study of Lapicque's theory of curarization.

2. When precautions are taken to avoid stimulating the intramuscular nerves in the uncurarized preparation by (*a*) using "block" electrodes and observing the α curve, or (*b*) using stigmatic electrodes applied to the nerve-free part of the muscle, curare is found to paralyse without affecting the strength-duration curve of the muscle in any way.

3. This is in complete agreement with the recent observations of Grundfest and with many former workers.

4. Lapicque's claim as to the antagonism of strychnine and veratrine was not confirmed. In no single case was curarization by one drug removed by the application of the other.

5. Lapicque's most recent argument in favour of his curarization theory relates to excitation by slowly increasing currents. This argument is criticized. His experiments lie open to the interpretation that curare has no effect upon muscle excitability; initially not muscle but the intramuscular nerve twigs are excited, and only after curarization is the muscle directly stimulated. That Lapicque's observed excitability change upon curarization is due to this transfer from nerve to muscle, is confirmed by the present experiments into which initial nerve excitation does not enter.

6. Thus there is positive evidence against Lapicque's theory of curarization, and no unequivocal evidence in its favour.

This paper completes the account of the experiments I made in the University of Pennsylvania, and I should like to express here my sincere appreciation of the welcome and assistance which I received in Philadelphia, and especially to thank Prof. Bronk and Mr A. J. Rawson.

It is also a pleasure to own my indebtedness to the Government Grants Committee of the Royal Society.

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THE EFFECT OF INSULIN ON THE RESPIRATORY QUOTIENT, OXYGEN CONSUMPTION, SUGAR UTILIZATION, AND GLYCOGEN SYNTHESIS IN THE NORMAL MAMMALIAN HEART IN HYPER- AND HYPOGLYCÆMIA.

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While attention has been largely focused on the action of insulin on the mammalian organism as a whole under conditions of hyperglycæmia or maintained blood-sugar levels, comparatively little attention has been directed to the action of insulin under hypoglycæmia conditions. The many problems of carbohydrate metabolism which have been opened up by the discovery of insulin still demand that the functions of the organs of the mammalian body be individually investigated as a logical step towards the elucidation of the action of this hormone upon the body considered as a whole.

The results to be presented and discussed here are the outcome of previous work upon the action of insulin on the normal and diabetic heart. In certain respects the diabetic heart does not apparently behave as does skeletal muscle and whether or not the normal heart should be expected to conform in all functional respects to skeletal muscle is still an open question. That one of the best means by which to determine the reaction of skeletal muscle to insulin is the use of the eviscerated spinal preparation as suggested by Burn and Dale [1924] and others few will deny; but that it is a good standard by which to compare the results of similar investigations upon cardiac muscle is a moot point. And further, it is essential that, in assessing the value of results obtained on cardiac muscle, one should not be obsessed by the findings recorded for skeletal muscle. Such investigation demands that the heart be allowed to function in as normal a condition as possible: to remove the heart from the body of an animal, wash it in saline or Locke-Ringer solution, remove adherent tissue from it, attach it to a perfusion apparatus and then perfuse it with Locke-Ringer solution or diluted blood is certainly not the best means of securing figures indicative of the normal reactions of that organ. Despite its disabilities the heart-lung preparation of Starling

is still of value; the preparation can be completed in 15 minutes, the heart suffers no handling, is supplied with its own blood, and the lungs assure an efficient oxygenation of the blood, and where the gaseous metabolism of the heart-lung preparation must be determined both the circulatory system and the respiratory system can be effectively closed.

Methods.

To arrive at an accurate idea as to the gaseous metabolism of the heart, oxygen and CO_2 determinations were carried out in a manner which will be briefly described.

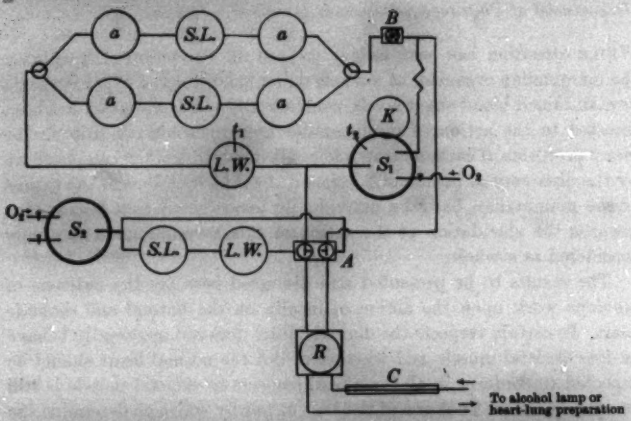

Fig. 1. Diagram of apparatus. *A*, stopcocks for connecting or disconnecting alcohol lamp or isolated heart-lung preparation from the recording system; *B*, rotary blower; S_1, spirometer with *K*, kymograph for record of oxygen consumption; S_2, reserve spirometer; *R*, respiration pump; *SL*, soda lime bottle; *a*, acid bottle; *LW*, lime water bottle; *C*, condenser; t_1, t_2, thermometers reading to 0·1° C.

Description of apparatus. An examination of Fig. 1 and its legend will show the general arrangement of apparatus. It will be seen that there are two spirometers each with a CO_2 absorbing system, with either of which the lungs of the animal may be connected by stopcocks *A*. In the case of a mammalian experiment this permits of efficient oxygenation of the blood with a removal of CO_2, thus allowing the blood CO_2 content to fall to a steady low level before commencing the experiment. It will be noted that when the animal is turned into the system associated with

spirometer S_1, no expired air can enter the spirometer. In order to ensure this, the tube marked *D*, leading from the spirometer to the rotary blower, is of 300 c.c. capacity. A large portion of the expired air passes into this tube before being passed on by the rotary blower *B*, simply because the velocity of the expiration due to the elasticity of the lungs with a respiration rate of eighteen per minute and a tidal air volume of 150 c.c. may be as high as 200 c.c. per sec. It is unnecessary to circulate the air at a rate of 28 to 30 litres per minute; 7 to 12 litres per minute is ample and allows of a complete absorption of CO_2.

The temperature is noted by thermometers reading to 0·1° C. in Williams's bottles, t_1, t_2, and in the cannula which passes the blood to the heart. Soda-lime is kept in Williams's bottles, not in the usual soda-lime bottles, which have been found unsatisfactory. When the Williams's bottles with soda lime and acid have been weighed and placed in position the rotary blower is started, and the air in the system with spirometer S_1 circulated for 15 minutes to bring it to a constant temperature. Meanwhile the lungs are being artificially inflated by a Starling Ideal pump placed in oil, the air being circulated through a CO_2 absorbing system and spirometer S_2 which has previously been filled with oxygen. When the temperature at t_1 is steady the lungs are switched into connection with S_1 by means of the stopcocks *A*, the kymograph and stop-watch are started and the temperatures noted. At any time the circulating air can be turned into the parallel absorbing system and a new period started.

Alcohol lamp check experiments on the apparatus. Absolute ethyl alcohol of 99·95 p.c. purity and having a sp.gr. of 0·794 was used. The oxygen equivalent of the alcohol was, 1 c.c. = 1159·60 c.c. of O_2. The factor for the large spirometer bell is calculated from the steel mould from which it is made. This mould had a diameter of 16·25 cm., which gives a factor of 20·73 c.c. per mm. excursion of the bell. For the estimation of small amounts of oxygen consumed by the heart we had made for us a very finely balanced spirometer, the bell of which is 7·55 cm. diameter and 34 cm. in height, having a factor of 4·461 c.c. per mm.

The system as described, and as shown in Fig. 1, was tested for leaks by recording movements of the spirometer bell for 15 minutes, in which time the recording kymograph drum made one complete revolution.

Respiratory quotients with the alcohol lamp. Reference to Table I will show the details of ten check experiments. The experimental error for the estimation of oxygen consumed averages 0·667 p.c., that for R.Q.'s averages 0·225 p.c. In the case of organs where the amounts of oxygen utilized per minute is very small it is possible to secure, with the smaller

spirometer, a continuous and very accurate record of oxygen utilization over periods extending from 1, 2 or more hours, depending upon the gradient of the curve of oxygen consumption.

TABLE I. R.Q. check experiments with alcohol lamp.

No.	Alcohol c.c.	Spirometer mm.	t_2 change °C.	t_1 change °C.	Theoretical O_2 c.c.	Exp. O_2 c.c.	Percentage O_2 rec.	CO_2 c.c.	R.Q. exp.
1	1·42	85	0·20	0·20	205·90	205·53	99·71	136·82	0·665
2	1·76	107	0·20	0·00	132·10	131·65	99·65	87·94	0·668
3	2·45	154	0·04	0·10	189·46	193·01	101·87	130·85	0·677
4	2·78	172	0·00	0·12	214·98	214·65	99·89	145·88	0·678
5	1·75	111	0·10	0·05	203·00	205·48	101·02	136·38	0·664
6	1·42	90	0·05	0·10	205·90	210·60	102·27	143·10	0·675
7	2·20	157	0·00	0·02	216·92	217·15	100·10	144·08	0·664
8	1·90	118	0·20	0·03	220·40	220·89	100·22	146·05	0·661
9	2·03	129	0·03	0·00	235·48	239·35	101·64	158·77	0·663
10	1·85	115	0·00	0·00	214·61	215·28	100·31	143·50	0·666

Time of experiments 10 min. by stop watch, except Nos. 1 and 6 = 8 min.
Nos. 2 and 3 = 15 „

Average R.Q. = 0·6681
Theoretical = 0·6666
Average p.c. O_2 recovered = 100·66

The isolated heart-lung preparation needs no description, suffice it to add that the reservoir, in which the blood is usually exposed to the external air, was effectively closed by being connected to a small spirometer filled with nitrogen. The fall in the bell of this spirometer indicated with extreme accuracy any alteration in the blood volume of the preparation.

Sugar estimations were made by the Shaffer and Hartmann [1920] method, lactic acid by West's modification of the method described by Friedemann, Cotonio and Shaffer [1927]. Numerous experiments have been carried out and, as these entail the presentation of many figures, only two or three typical examples of each will be given. For convenience in discussing the subject-matter the work will be presented under the following divisions.

Part I. *Hyperglycæmia with and without insulin.*

(1) The utilization of sugar.
(2) The respiratory quotient and oxygen consumption.
(3) The synthesis of glycogen.

Part II. *Hypoglycæmia with and without insulin.*

(1) The utilization of sugar.
(2) The respiratory quotient and oxygen consumption.
(3) The utilization or synthesis of glycogen.

Part I. Hyperglycæmia.

(1) *The utilization of sugar.*

In discussing the question of sugar utilization it is essential not to confuse sugar utilization by the heart with the amount of sugar disappearing from the circulating blood. The early experiments of Locke and Rosenheim [1907], Knowlton and Starling [1912*a*], Mansfeld [1914], Patterson and Starling [1913], and later experiments of Hepburn and Latchford [1922], Burn and Dale [1924], of Cruickshank and Shrivastava [1930] and of many others have shown that the mammalian heart removes sugar from the blood, the figures, under varied experimental conditions, ranging from 2 to 6 mg. per g. of heart muscle per hour. In using the isolated heart-lung preparation to determine sugar utilization by the heart, Starling and Evans [1914] allowed 1·3 mg. per g. of heart per hour for the sugar consumption of the lungs and the blood. Cruickshank and Startup [1930] place the sugar consumption of the dog's lungs at 0·7 to 0·8 mg. per g. of heart muscle per hour. The rate of disappearance of sugar from circulating well-oxygenated blood has been investigated by Cruickshank and Startup [1932] and they have shown that a progressive oxidation of sugar takes place amounting to approximately 10 p.c. of the total blood sugar in the first hour and about 14 p.c. in the second hour. No glycolysis takes place in this time, provided oxygenation is good. It would seem, therefore, from these facts that a correction of 1 mg. per g. of heart muscle per hour should be made for utilization of the lungs in determining absolute figures for the sugar usage of the heart. This correction has not been made in the tables presented in this paper.

In hyperglycæmia without insulin the utilization of sugar is increased from an average normal of 5·28 to 5·84 mg. per g. of heart muscle per hour. The addition of insulin increases the sugar consumption to the average figure of 6·06 mg. per g. per hour. In these experiments the blood-sugar level was never in excess of 0·218 g. p.c. Where the sugar content is increased to 0·40 g. p.c. or more there is not a proportional increase in the utilization under insulin. The addition of sugar to the blood whereby a moderate condition of hyperglycæmia is maintained raises sugar oxidation 10·5 p.c.

The effect of insulin. The addition of ten units of insulin per hour has a much less effect than excess sugar in stimulating sugar oxidation (Table II); the average percentage increase being 3·62. In any discussion of sugar utilization, when the disappearance of blood sugar is in question,

TABLE II. Hyperglycæmia.

The effect of hyperglycæmia and of added insulin upon the R.Q. oxygen consumption and sugar oxidation.

Hour of exp.	Blood sugar g. p.c. Begin	Blood sugar g. p.c. End	CO_2 c.c.	O_2 c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr.	Lactic acid mg. p.c. Begin	Lactic acid mg. p.c. End	
1	0·1128	0·0659	362·29	357·71	1·012	4·47	5·07	31·5	28·8	Normal
2	0·1701	0·1419	381·68	375·67	1·016	4·70	6·25	31·5	27·0	Insulin
1	0·1432	0·1008	302·51	273·40	1·106	4·43	5·78	54·9	55·8	Normal
2	0·1863	0·1244	279·89	283·50	0·980	4·50	6·00	55·8	58·5	Insulin
1	0·1708	0·1206	361·31	363·45	0·999	4·33	5·78	27·2	28·8	Normal
2	0·2218	0·1244	369·99	375·40	1·018	4·46	5·93	31·5	27·9	Insulin
Average										
	—	—	—	336·60	—	3·96	5·28	Normal, 6 exps.		
	0·1422	0·1039	342·04	331·55	1·039	4·376	5·84	With sugar		
	0·1927	0·1319	343·85	344·85	1·004	4·553	6·06	With insulin		
	Increase due to sugar					10·50	10·50			
	Increase due to insulin					3·62	3·62			

one must clearly discern between oxidation and synthesis. As this involves a discussion of both sugar and oxygen consumption in the presence of insulin and also of the direct action of insulin on the heart muscle, the matter will be fully referred to under the section dealing with the effect of insulin upon the oxygen consumption of the heart in hypoglycæmia.

(2) *The respiratory quotient and oxygen consumption.*

The normal isolated heart in the presence of an adequate supply of blood sugar has always given a R.Q. which is unity. Bayliss, Müller and Starling [1928] find a R.Q. for the isolated heart of 0·90, a figure determined after 3 hours have been spent upon the operation and on the setting up of the apparatus. In our experiments the preparation is completed in 15 to 20 minutes and runs for periods of 1, 2 or 3 hours. The average figures for the utilization of oxygen (Table II) show how small is the effect, first of added sugar and second of sugar plus insulin. With a maintained blood sugar the heart consumes approximately 4 c.c. O_2 per g. of heart muscle per hour. Upon the addition of sugar, raising the percentage to an approximate level of 0·15 g., the oxygen consumption per g. per hour rises to an average of 4·376 c.c. while the subsequent addition of ten units of insulin raises the figure to 4·553 c.c. per g. per hour, an increase of 3·62 p.c. As with added sugar so with insulin; the increase in oxygen consumed is determined by the amount

of sugar utilized. Figures for the lactic-acid content of the blood show that changes in the production of lactic acid are of such a small order in the first experimental hour that they cannot materially alter the results obtained on the oxygen consumption and sugar utilization by the isolated heart in hyperglycæmia.

(3) *Glycogen synthesis.*

This was determined by correlating the blood sugar lost with the sugar equivalent of the oxygen consumed.

With the maintenance of the blood-sugar level above the normal there is a slight increase in the deposition of glycogen in the heart. Upon the addition of insulin there is a marked increase in the amount of sugar deposited as glycogen in the heart. Table III shows that of the

TABLE III. Hyperglycæmia.

Effect of insulin on the absolute and percentage amounts of blood sugar oxidized and stored by the mammalian heart.

(Blood-sugar percentages and R.Q.'s are shown in Table II.)

Exp.	Total sugar lost g.	Sugar oxidized g.	Sugar stored g.	Percentage blood sugar oxidized g.	Percentage blood sugar stored g.	Wt. of heart g.	Possible glycogen in heart g.	Percentage increased in heart glycogen	
I	0·4907	0·4769	0·0138	97·2	2·8	80	0·400	3·5	Normal
	0·6405	0·5008	0·1397	78·2	21·8		0·414 0·553	35·0	Insulin
II	0·3752	0·3644	0·0108	97·2	2·8	63	0·315	3·1	Normal
	0·5054	0·3780	0·1274	74·8	25·2		0·325 0·452	39·1	Insulin
III	0·5004	0·4858	0·0146	97·1	2·9	84	0·420	3·3	Normal
	0·6564	0·5005	0·1560	76·4	23·6		0·434 0·590	36·2	Insulin

The percentage increase in the oxidation of sugar and the deposition of glycogen in the heart in hyperglycæmia with and without insulin (Tables II and III).

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Glycogen	Oxidation	Glycogen
10·5	3·30	3·62	36·70

The effect of hyperglycæmia with and without insulin upon the balance between the oxidation and synthesis of 100 parts of blood sugar.

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Synthesis	Oxidation	Synthesis
97·2	2·8	76·1	23·5

total amount of sugar which has disappeared from the blood, the amount deposited has increased from 2·8 to 23·5 p.c. These results are even more striking when we consider the relation between the total amount of blood sugar used for oxidation and that used for the synthesis of glycogen. Taking average figures; the amount of sugar oxidized increases from 0·4433 to 0·4736 g., an increase of 0·0313 g., that used for the synthesis of glycogen increases from 0·0131 to 0·1442 g., an increase of 0·1311 g. The former figure shows a negligible increase, the latter a tenfold increase.

If we assume that the heart muscle has an initial glycogen content of 0·500 g. p.c., estimated as sugar, we see from Table III that there has been an average increase from 3·3 to 36·7 p.c. These figures for glycogen deposition, which are in terms of the original amount of glycogen in the heart, are in remarkable agreement with the findings of Cruickshank and Shrivastava [1930] who found, by numerous direct estimations of heart glycogen in dogs, that at the end of 2 hours' insulin administration with a blood sugar not greater than 0·222 g. p.c., there was an average change from 0·535 to 0·719 g., a 34·3 p.c. increase.

From a consideration of such results it is fair to conclude that insulin has, as far as cardiac muscle is concerned in the presence of hyperglycæmia, a very marked stimulating effect upon glycogen synthesis, and plays but a small part in increasing sugar oxidation. It is also of interest under these conditions to note the effect of insulin on the percentage distribution of blood sugar between oxidation and synthesis. Normally the ratio is 97·2 to 2·8; with insulin it becomes 76·1 to 23·5, a ratio which is noteworthy, as will appear later.

Part II. Hypoglycæmia.

(1) *Utilization of sugar.*

It may be inferred that the heart is faced with a condition of hypoglycæmia when the blood sugar is not sufficient to supply fully the energy requirements of the organ, and as a result thereof a certain amount of glycogen has been utilized. This condition of affairs invariably happens when the blood sugar has been reduced to 0·05 g. p.c. In an isolated heart-lung preparation with a blood sugar of 0·0935 g. p.c. at the beginning and 0·0546 g. p.c. at the end of 1 hour's experiment, the amount of sugar utilized averages 4·55 mg. per g. of heart muscle per hour (Table IV). But the amount of sugar disappearing from the blood is not sufficient for the energy needs of the heart, with the result that there is a call upon the glycogen content of the heart. It can be seen from Table V that the

Table IV. Hypoglycæmia.

Effect of hypoglycæmia upon the R.Q., the oxygen consumption and sugar oxidation in the mammalian heart. Time = 1 hour.

Blood sugar g. p.c.		CO_2	O_2		Oxygen c.c. per g.	Sugar oxidized mg. per g. per hr.	Lactic acid mg. p.c.	
Begin	End	c.c.	c.c.	R.Q.	per hr.	g.	Begin	End
0·0979	0·0572	278·89	280·35	0·992	3·50	4·67	40·5	43·6
0·0928	0·0599	336·07	339·12	0·991	3·02	4·04	45·0	49·5
0·1121	0·0620	473·14	468·00	1·011	3·60	4·80	36·0	41·4
0·1009	0·0712	549·60	554·41	0·996	3·46	4·62	37·8	42·3
0·1019	0·0488	509·79	524·37	0·972	3·49	4·66	57·6	59·4
Average								
0·0935	0·0546	434·10	425·61	0·992	3·42	4·55		
Normal of 6 expts.		—	336·60	—	3·96	5·28		
Percentage fall below normal			—	—	13·90	13·90		

Table V. Hypoglycæmia.

Effect of hypoglycæmia on the glycogen content of the heart. (Blood-sugar percentages and R.Q.'s are shown in Table IV.)

Exp.	Blood sugar lost g.	Total sugar oxidized g.	Percentage sugar added to blood sugar for oxidation	Glycogen lost g.	Weight of heart g.	Possible heart glycogen g.	Percentage glycogen lost
I	0·2584	0·3738	44·6	0·1154	80	0·400	28·8
II	0·3342	0·4521	30·9	0·1184	112	0·560	21·1
III	0·5608	0·6240	11·3	0·0636	130	0·650	9·7
IV	0·5961	0·7392	23·9	0·1431	160	0·800	17·8
V	0·4828	0·6858	42·0	0·2030	150	0·750	27·0

Sugar added to blood sugar from heart reserves = 30·54 p.c.
Average loss of glycogen in first hour = 20·88 p.c.

blood sugar used has been augmented 11·3 to 44·6 p.c. by a call upon the carbohydrate reserve of the heart muscle. It is, of course, impossible to determine the glycogen content of the heart at the commencement of the experiment, but it is of interest to note the loss or gain of glycogen assessing the original heart glycogen from the assumption that the normal heart contains 0·500 g. p.c. of its weight as glycogen. It is an assumption based on the average results of numerous experiments dating from the year 1912; reference has already been made to the close parallel between these figures and those obtained from direct estimation of heart glycogen. On this assumption it is seen that there is a glycogen loss varying in five experiments from 9·7 to 28·8 p.c., with an average loss of 20·88 p.c. From such results one is led to the conclusion that the heart, when faced with a slight hypoglycæmia, draws upon its carbohydrate reserves

and, as will be seen later when the R.Q.'s are discussed, upon nothing else, at least not as far as can be judged from an experiment lasting only 1 hour. This is not in accord with what obtains in the eviscerated spinal cat, where a slight degree of hypoglycæmia is not accompanied by any marked loss in the glycogen of the skeletal muscle [Best, Hoet and Marks, 1926]. In the eviscerated preparation, however, liver glycogen may have been available, the blood-sugar level was never lower than 0·068 g. p.c., and no estimation of heart glycogen was made.

The effect of insulin. It has been seen that, when no sugar is added to maintain a constant blood-sugar level, the sugar utilization falls from the

TABLE VI. Hypoglycæmia.

The effect of insulin on the R.Q., oxygen consumption, and sugar utilization of the heart.

Hour of exp.	Blood sugar g. p.c. Begin	Blood sugar g. p.c. End	CO_2 c.c.	O_2 c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr.	Insulin units	Lactic acid mg. p.c. Begin	Lactic acid mg. p.c. End
1	0·1024	0·0592	356·23	353·63	1·007	4·21	5·61	10	34·2	36·9
2	0·0592	0·0246	346·05	360·50	0·960	4·29	4·74	10	36·9	43·2
3	0·0246	0·0246	277·08	357·48	0·775	4·25	1·42	10	43·2	40·5
1	0·1091	0·0605	381·57	352·30	1·082	4·14	5·52	10	45·0	40·5
2	0·0605	0·0447	305·34	356·40	0·860	4·20	2·85	10	40·5	36·9
3	0·0447	0·0313	264·44	356·11	0·744	4·20	0·72	10	36·9	22·5
1	0·0977	0·0413	356·23	364·50	0·988	4·18	5·58	10	27·0	27·0
2	0·0413	0·0261	305·34	380·75	0·802	4·38	1·83	10	27·0	24·0
1	0·1009	0·0712	549·60	554·41	0·996	3·46	4·62	0	37·8	42·3
2	0·0712	0·0506	503·80	574·12	0·877	3·58	2·77	10	42·3	25·2
1	0·1019	0·0488	509·79	524·37	0·972	3·49	4·66	0	57·6	59·4
2	0·0673	0·0315	492·13	550·88	0·910	3·66	3·47	10	54·9	41·4

Note. (1) In the two last experiments insulin was given at the commencement of the second hour.

(2) The amount of sugar oxidized for R.Q. given has been determined from the tables of Zuntz and Schumberg.

Average figures.

Normal of 6 expts.	336·60	—	3·96	5·28

Average weight of heart = 85·4 g.

Hypoglycæmia with insulin from the beginning of the experiment.

First hour:			
Commencing blood sugar normal	1·028	4·17	5·56
Second hour:			
With very low blood sugar	0·881	4·30	3·09
Third hour:	0·760	4·24	1·07

Percentage changes in the amount of oxygen used and sugar oxidized in hypoglycæmia with insulin.

	Oxygen used	Sugar oxidized
First hour	+5·30	+ 5·30
Second hour	+3·12	−44·42

normal of 5·28 to 4·55 mg. per g. per hour, a depression of 13·90 p.c. The addition of insulin at the beginning of the experiment when the blood sugar is normal results in a slight increase of sugar utilization to 5·56 mg. per g. per hour, an increase of 5·30 p.c. (Table VI). If, however, the insulin be added at the end of the first hour of the experiment, when a definite degree of hypoglycæmia has become established, the effect is a decrease in sugar consumption as shown in the last two experiments in Table VI. This is corroborated by what obtains when insulin is added hour by hour; the amount of sugar used becomes less and less, until finally little sugar if any may be utilized. Table VII shows this result; after a slight initial rise in the first hour, sugar utilization falls 44·42 p.c.

TABLE VII. Hypoglycæmia.

The effect of insulin on the oxidation and storage of blood sugar by the mammalian heart. (Blood-sugar percentages and R.Q.'s as in Table VI).

Hour of exp.	Total sugar lost g.	Blood sugar oxidized g.	Blood sugar stored g.	Percentage of sugar oxidized	Percentage of sugar deposited g.	Weight of heart g.	Possible heart glycogen g.	Percentage gain heart glycogen
1	0·5332	0·4712	0·0620	88·38	11·62	84	0·420	14·80
2	0·4482	0·3985	0·0497	88·91	11·09	—	0·482 0·532	10·31
1	0·5263	0·4696	0·0567	89·23	10·77	85	0·425	13·34
2	0·2475	0·2204	0·0271	81·52	19·48	—	0·481 0·508	5·58
1	0·5367	0·4666	0·0701	86·94	13·06	87	0·435	16·10
2	0·1788	0·1589	0·0199	88·78	11·22	—	0·506 0·524	3·54
2	0·5246	0·4436	0·0810	84·56	15·44	160	0·656 0·737	12·34
2	0·5877	0·5210	0·0667	88·65	11·35	150	0·547 0·614	12·19

The percentage changes due to hypoglycæmia with and without insulin in the oxidation processes and the deposition of glycogen in the heart (Tables VI, VII).

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Glycogen lost	Oxidation	Glycogen gained
First hour	−13·90	−20·88	5·30	13·75
Second hour	—	—	3·12	6·48

The effect of hypoglycæmia with and without insulin upon the ratio between the oxidation and synthesis of 100 parts of blood sugar.

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Synthesis	Oxidation	Synthesis
First hour	100·00	0·00	87·55	12·45
Second hour	—	—	86·17	13·93

in the second hour and 66·11 p.c. in the third hour. Third-hour results have not been included in the tables because the onset of lung œdema makes them unsatisfactory. When the blood sugar is reduced to about 0·025 g. p.c. the heart has apparently ceased to make use of sugar as its chief source of energy. That there is a condition of glucatonia is clear from the very small amount of sugar in the serum at the end of the third experimental hour. Such a diminution of sugar in the serum points to an extremely small amount of free muscle sugar, proof of a thorough removal of sugar by the heart. The question arises now as to whether the sugar so removed has been oxidized or synthesized.

(2) *The respiratory quotient and oxygen consumption.*

The respiratory metabolism of the isolated mammalian heart can best be investigated by the isolated heart-lung preparation of Starling [Knowlton and Starling, 1912*b*], for here the heart can be kept beating powerfully and efficiently for several hours with both the circulating and respiratory systems closed from the external air.

"If the heart muscle, like skeletal muscle, performs its work by oxidising preformed carbohydrate exclusively then the respiratory quotient should be unity" [MacLeod, 1928]. Lovatt Evans [1912] found great variations in the R.Q. of mammalian hearts, the average for dogs being 0·901. Starling and Evans [1914] obtained quotients as low as 0·70 and as high as 1·09 with an average of 0·85. It has been assumed, because of such results, that important metabolic differences exist between skeletal and cardiac muscle.

A high R.Q. may be due to the washing out of CO_2 in such experiments [Kilborn, 1928], but this factor has been controlled in the experiments discussed here by adjusting the extent of ventilation to the size of the preparation [Corkhill, Dale and Marks, 1930; Eggleton and Evans, 1930], maintaining a much more moderate degree of ventilation than is usual in such experiments and by allowing at least half an hour for complete gaseous equilibrium to be obtained before commencing the actual experiment. With a ventilation rate which was generally constant at twelve strokes per minute, the stroke volume, which varied between 50 and 100 c.c., was changed in accordance with the size of the heart and lungs. Over-ventilation was thus guarded against, and the small amount of tissue used permitted of a fairly quick arrival at gaseous equilibrium. No anoxæmia was ever present, a perfect oxygenation of the blood was always in evidence to the end. That there was no failure in oxidative processes in the preparation is also seen from the fact that there was little

increase of lactic acid in the blood, in fact, in the insulin experiments there was usually a marked diminution in the lactic acid content of the blood.

The effect of hypoglycæmia per se *on the respiratory metabolism of the heart.* Table IV shows that the R.Q. for the first experimental hour is essentially unity, the highest figure being 1·011, the lowest 0·991, with an average of 0·992. A moderate degree of hypoglycæmia has therefore had no effect upon the type of metabolic activity. The oxygen consumption is also without marked variations when reduced to c.c. per g. of heart muscle per hour. The lactic acid of the blood varied in these experiments from 36 to 59 mg. p.c. The variation in each experiment is so small as to be regarded as negligible as far as any change in oxidative processes may be concerned. Hypoglycæmia produced a diminution in the utilization of oxygen from 3·96 to 3·42 mg. per g. of muscle per hour, an average fall of 13·90 p.c.; sugar utilization has also been reduced to a similar extent from 5·28 to 4·55 mg. per g. of heart muscle per hour. The normal figures are from the records of six experiments in which the sugar level was maintained by the use of a Master's constant injection pump.

The effect of insulin. It has been shown that, with an increase in the sugar content of the blood above the normal level for the animal, there is a slight increase in oxygen consumption. The addition of insulin in hyperglycæmia effects a further slight increase in the oxidation of sugar by the heart, but the oxygen absorbed does not account for all the sugar which has disappeared; much of it has been retained as glycogen. Experiments by Burn and Dale [1924] upon eviscerated decapitated cats led them to the conclusion that the earlier stages of insulin action are accompanied by increased consumption of oxygen in a preparation, the R.Q. of which was always about unity.

In a later paper Best, Dale, Hoet and Marks [1926*a*] state that there is always a slight depression of respiratory metabolism following insulin administration which, calculated from only one experiment, amounts to 5·4 p.c. of the oxygen figure for the normal period, and they say that "the excess oxygen consumption observed by Burn and Dale [1924] can be entirely accounted for by the contribution to the blood from the carbohydrates of the liver." But a recalculation of Burn and Dale's results will show that, after allowing for the leakage of sugar, the increased amount oxidized in the presence of insulin varies from 9·1 to 43·8 p.c. with an average figure of 23·4 p.c. or, leaving out the extreme figure of 43·8 p.c., with an average figure of 16·3 p.c. Experiments of Chaikoff and MacLeod [1927] have shown that insulin causes a relatively slight increase in the oxidation of carbohydrates in the

rabbit but a definite increase in the isolated perfused muscles of the cat. They also point out that in dogs there is also a very slight increase in oxygen consumption when insulin is administered with glucose. A marked increase in oxygen consumption by animals to which insulin has been given has been thought to be due to a condition of hyper-excitability of muscle tissue which preceded the onset of convulsions [Dickson, Eadie, MacLeod and Pember, 1924]. On the other hand, Visscher and Müller [1927] have demonstrated that non-pressor samples of insulin produce no direct stimulation of oxidative metabolism in the heart.

It is clear from what has been said that one should determine whether or not insulin has any direct effect upon the preparation used, be it a whole animal or an isolated tissue. Since the discussion here is one of synthesis versus oxidation as characteristic of insulin action, pressor effects must either be eliminated or accounted for. In the isolated heart-lung preparation the heart suffers no change in rate when twenty units of insulin are added to the blood; there is, however, a slight rise in blood-pressure which never exceeds 10 mm. Hg, and which passes off within 10 to 15 minutes. Further it is seen that there is, during this time, a slight increase of 20 to 30 c.c. in the volume of blood in the reservoir, which is due to a reduction in the blood volume of the preparation, the volume returns to its pre-insulin level within 15 minutes. Here then is evidence of a slight and transient pressor effect upon isolated heart muscle. To make a correct allowance in the figure for oxygen consumption for this pressure change one must take into consideration its duration. A 10 p.c. rise in pressure disappearing in 10 minutes would necessitate a correction for the figures given of approximately 1·7 p.c. This correction has not been made in the tables.

Experiments were carried out on the whole animal (two dogs and three cats) to see to what extent oxygen consumption would be increased in the presence of forty units of insulin. In the cats the oxygen consumption increase averaged 7·69 p.c. in 1 hour, there was a very slight increase of 5 mm. Hg in blood-pressure, the increase in heart rate averaged 1·8 p.c. In the dogs there was no change in blood-pressure, an average increase in heart rate of 1·7 p.c. and an average increase in oxygen consumption of 2·7 p.c. A repetition, within half an hour of the first dose, of forty units of insulin caused a slight fall in blood-pressure. Pressor effects due to the insulin can therefore be regarded as negligible. Dr K. K. Chen of Eli Lilly and Co. very kindly tested all the samples of insulin which he sent to us and found that they caused no rise of blood pressure in the pithed cat.

Insulin given in ten unit doses hourly in hypoglycæmia raises oxygen consumption 5·30 p.c. in the first hour (Table VII). In the second hour there is a continued slight rise in oxygen consumption, and in the third hour there is no appreciable change. Further, insulin addition generally produces a definite fall in the lactic-acid content of the blood which, with a slight increase in oxygen used, a decrease in CO_2 produced, and a fall in the R.Q. would indicate that the lactic acid is not oxidized but is reconverted to glycogen. The slight increased oxidation must therefore be attributed to a consumption by the heart of non-carbohydrate substances. From such results one must conclude that the stimulation of oxidative processes is not essentially a characteristic of insulin action.

The most noteworthy change, however, is that seen in the R.Q. A fall in the R.Q. from 1·00 to 0·744 (Table VI) indicates a definite change in metabolism. As no measure of the extent of protein metabolism has been obtained and as a correction at a R.Q. of 0·82 would entail approximately a 10 to 15 p.c. change which would not materially affect the results as set down, the R.Q.'s have been taken as representing the balance between carbohydrate and fat in so far as figures for the oxidation of sugar have been given. The relative proportion of the oxygen consumed by sugar has been obtained from the well-known tables of Zuntz and Schumburg. It may be taken, from a consideration of the fall in the R.Q.'s, that the energy cost of the work that is being done by the heart is greater since the calorific output as estimated by oxygen consumption is slightly raised and maintained over a period of 3 hours, and the metabolic activity of the heart muscle has changed in type, namely from a purely carbohydrate source of energy to one consisting to a large extent of non-carbohydrate material.

(3) *The utilization or synthesis of glycogen.*

It has been clearly shown by Best, *et al.* [1926*a*] and Burn and Dale, [1924], Cori, Cori and Pucher [1923], Bissinger, Lesser and Zipf [1923], Cruickshank and Shrivastava [1930] that the chief action of insulin is to cause increased glycogen formation in the presence of an adequate supply of blood sugar. The observations of Bissinger, *et al.* and of McCormick and MacLeod [1923] showed that, while glycogen is deposited as an early action of insulin, little if any excess remains after a certain period. Dudley and Marrian [1923] concluded as a result of their experiments that hypoglycæmia due to insulin produced a definite glycogenolysis in liver and muscle but an increase in glycogen within the heart muscle. It has, however, been clearly shown [Best, Hoet and

Marks, 1926*b*] that hypoglycæmia with or without insulin does not, unless convulsions supervene, produce any breakdown in the glycogen of skeletal muscles. In the eviscerated spinal preparation the blood sugar is evidently sufficient for the energy needs of the preparation, with the result that a certain small amount of glycogen is stored. Upon the addition of insulin with a blood-sugar level at the end of the experiment of 0·06 g. p.c. there is an increase in glycogen to the extent of 28 p.c. [Best, *et al.* 1926*a*].

Reference has already been made to the loss of heart glycogen occasioned by a marked hypoglycæmia, namely an augmentation of blood sugar from heart glycogen averaging 30·54 p.c. or an average of 20·88 p.c. of the calculated glycogen content of the heart being utilized. This loss is progressive and may with a continued hypoglycæmia be as much as 30 p.c. of available glycogen at the end of 2 hours. It would be of interest to determine the ultimate reaction of the isolated mammalian heart when faced with practically no blood sugar, and a 50 or 60 p.c. depletion of its glycogen.

The effect of insulin. The results shown in Table VII indicate that in insulin hypoglycæmia lasting 2 to 3 hours there is such a depletion of blood sugar that further sources of energy are necessary. It should also be remembered that the depletion of sugar may be greater than the figures would indicate, because the method of estimation of blood sugar has not excluded non-carbohydrate reducing substances [Somogyi, 1930]. In the first hour, with a R.Q. of unity, of the total amount of blood sugar which has disappeared, an average of 12·5 p.c. has been deposited in the heart muscle, in the second hour 13·93 p.c. In terms of the assumed original amount of glycogen in the heart the increase in glycogen is, in the first and second hours, 13·75 and 6·48 g. p.c. This shows that in a progressive insulin hypoglycæmia while the blood is rendered almost sugar free, and the amount of glycogen deposited in the heart has accordingly fallen, yet the ratio distribution between sugar oxidized and sugar synthesized is remarkably well maintained. It is apparent that insulin effects a balance between these two activities and that in favour of synthesis, and further, that it continues to do so when the heart exposed to an extreme degree of hypoglycæmia is forced to utilize other substances as sources of energy.

It is thus demonstrated that, what can be easily obtained by skeletal muscle under much more favourable conditions than those afforded the isolated heart, has been attempted, and that with considerable success, by cardiac muscle.

Discussion.

It would appear from such results that, while carbohydrate is available to meet the increased energy needs of the heart muscle, carbohydrate only is used, but, that with the maintained energy requirements and with a rapid depletion of sugar supplies the heart muscle is forced to fall back on other sources of energy. That in its early stages exercise may be accomplished by the metabolism of carbohydrate has been shown by Hill [1925] and his co-workers, and that strenuous and continued exercise demands that the musculature of the body fall back on fat has been shown by Furusawa [1926].

It has been suggested by Greene [1926], from observations on the glycogen content of the muscle of salmon, that carbohydrate is not the immediate fuel of muscular activity; but that carbohydrate is the chief source of the energy of muscular contraction has been demonstrated by the work of Meyerhof [1924] and Hill [1922, 1932]. Burn and Dale [1924] found that the skeletal muscle of the eviscerated preparation before and after insulin had a R.Q. close to unity, and suggested therefrom that carbohydrate is the chief source of energy of isolated muscle.

On the other hand Kilborn [1928] offers no support to the theory that carbohydrate is the only source of energy for muscle contraction, and he attributed the high R.Q. of Burn and Dale to a washing out of CO_2. Bornstein [1929] showed that, in the eviscerated dog, a R.Q. of unity was maintained provided the blood-sugar level was kept up, but that it fell with a diminution in the carbohydrate supply. Corkhill, Dale and Marks [1930] have more critically investigated the question of over-ventilation as affecting the R.Q. in the eviscerated cat, and they state that a R.Q. of unity should be regarded as correct for that preparation provided the blood sugar is maintained at the normal value, and further they affirm that such a quotient cannot be regarded as artificially produced by excessive ventilation.

Meyerhof and Boyland [1931] have shown that skeletal muscle poisoned with iodoacetic acid and having a R.Q. of 0·7 to 0·8 recovers in oxygen with no lactic acid and with but little carbohydrate oxidation. The observations of Meyerhof [1931] and those of Witting, Markowitz and Mann [1930], on the isolated rabbit's heart perfused with glucose-free Ringer-Locke's solution, which show that an almost glycogen-free heart will beat as long as the normal perfused heart, would tend to strengthen the view that the work of muscular contraction may be performed by the oxidation of material other than carbohydrate. More

recent work by Clark, Gaddie and Stewart [1932] shows that frog's heart perfused for 6 hours under aerobic conditions gives a R.Q. of 0·87, from which they conclude that carbohydrate and protein would form 40 and 60 p.c. respectively of the total metabolism.

The results of our experiments on cardiac metabolism under hyperglycæmia and hypoglycæmia lead us to the conclusion that as with skeletal muscle so is it with mammalian heart muscle; given an adequate supply of carbohydrate the R.Q. will be in the region of unity, with a diminution of carbohydrate supplies the R.Q. will accordingly fall, indicating a definite change in the type of metabolism. While the question of fat and protein utilization is beyond the scope of this paper, still it would appear that when carbohydrate is not readily available as in hypoglycæmia then the heart must needs fall back on protein and fat, a view in keeping with the newer ideas as put forward by Embden and Meyerhof on the chemistry of skeletal muscle contraction.

It is further evident that, for the increase of glycogen in the heart in hyperglycæmia as for its restoration in hypoglycæmia, insulin is essential. This conclusion is in keeping with results obtained on skeletal muscle [Dale and Burn, 1924; Debois, 1930, 1931] and on dehepatized animals [Markowitz, Mann and Bollman, 1929]. It makes more difficult of explanation the fact that the diabetic heart contains more glycogen than the normal [Cruickshank, 1913] and that insulin does not lead to any increase in glycogen in the diabetic heart [Cruickshank and Shrivastava, 1930]. It is probable that glycogen synthesis is dependent upon a balance between sugar concentration and insulin activity.

The addition of insulin increases the call upon the blood sugar and, were it not for the fact that the function of synthesis is intensified by insulin, it is possible that the carbohydrate reserve of the heart would be the more readily available. While there is here no evidence for the assumption it may also be that insulin has stimulated gluconeogenesis from protein and fat within the heart muscle.

It would appear therefore from these experimental results that the immediate effect of insulin is to raise, very slightly, oxygen consumption and to increase markedly glycogen synthesis and to do so at the expense of blood sugar. This conclusion is inevitable in view of a R.Q. of unity and a definite degree of glycogen synthesis as shown in Tables VI and VII.

The continuance of insulin with the production of a definite hypoglycæmia, while it maintains oxygen consumption and glycogen synthesis, is associated with a marked fall in the amount of blood sugar utilized. With a fall in the R.Q., to an average of 0·879 and a reduction of 44 p.c.

in sugar utilization, it must be concluded that substances other than carbohydrates have been called upon to supply the energy requirements of the cardiac muscle. In the third hour blood sugar is practically depleted and glycogen continues to be stored, from which it is clear that there is a sparing of the carbohydrate supplies of the heart effected by the synthetic action of insulin. When available blood sugar has been reduced to an almost negligible quantity by insulin and the R.Q. has fallen to 0·760, the heart still endeavours to maintain an approximately normal oxygen consumption with protein and fat forming the great part of the fuel. And, further, from the fact that glycogen is synthesized it must be concluded that the synthetic action of insulin has far outweighed its action as a stimulant of oxidative processes: in fact the synthetic action has dominated the picture throughout, be it in hyperglycæmia or in hypoglycæmia.

Summary.

A method for the more accurate estimation of the R.Q. of the isolated heart has been described.

The gaseous metabolism, the sugar utilization, and glycogen synthesis of the normal heart have been investigated, with a maintained normal blood sugar, in hyperglycæmia and hypoglycæmia, with and without insulin.

Under maintained normal conditions of blood-pressure and blood sugar, the R.Q. is unity, the oxygen consumption is 3·960 c.c. per g. of heart muscle per hour, blood-sugar utilization being equivalent to the oxygen consumed.

Hyperglycæmia is always associated with an increase in oxygen consumption, sugar utilization, and glycogen synthesis.

The effect of hypoglycæmia *per se* on the normal heart has been investigated and it has been shown that, despite a definite depression in the general metabolic activity of the heart, and a progressive loss of glycogen, carbohydrates remain the only source of energy as long as carbohydrate is readily available.

The effect of insulin upon the metabolism of the heart in hyper- and hypoglycæmia has been studied, and it has been shown that, while in hyperglycæmia insulin increases oxygen consumption and sugar utilization to a very small extent, it markedly increases the synthesis of glycogen in heart muscle.

In the presence of a definite hypoglycæmia produced by insulin, the heart, with a R.Q. of unity, shows little increase in oxygen consumption,

but a marked increase in glycogen synthesis. With a progressive and marked reduction in the blood sugar the R.Q. of the heart steadily falls, indicating a progressive increase in the use of protein and fat as sources of energy, sugar utilization diminishes rapidly while glycogen synthesis is maintained. With a continued administration of insulin, the percentage relation between oxidation and synthesis tends to be changed towards the hyperglycæmic insulin ratio, namely from 97 : 3 to 86 : 14.

It has been demonstrated, in so far as the carbohydrate metabolism of cardiac muscle is concerned, that the essentially characteristic action of insulin is that of a stimulant of synthetic processes and not of oxidative metabolism.

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THE EXCRETION OF PROTEIN BY THE MAMMALIAN KIDNEY.

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It has been frequently stated in the literature of the last hundred years that certain proteins are excreted by the healthy mammalian kidney when they are introduced into the blood stream. The purpose of the present research was to investigate this property of the kidney, which was puzzling when considered according to current ideas of renal function. The excretion of gelatin, Bence-Jones protein, egg albumin, hæmoglobin, serum proteins of various animals, edestin, caseinogen and hæmocyanin was studied. Experiments were made both on anæsthetized animals and on isolated perfused kidneys. Histological examinations of the kidneys were made in a certain proportion of the cases.

Technique.

Cats and a few rabbits were anæsthetized with amytal, urethane or dial. The urine was collected by means of urethral cannulæ, the bladder being emptied and tied off above the level of entry of the ureters. Injections were made into an external jugular vein, and the blood-pressure was recorded from a carotid artery. The urine obtained from the bladder was always tested for protein at the beginning of the experiment.

Dogs were used for the isolated perfused kidney experiments, which had the advantage that possible complications arising from the influence of the added proteins on the general circulation were avoided. Pump-lung-kidney preparations were employed, similar to those used by Bayliss and Lundsgaard [1932], but with two modifications.

(1) In the pump, the oil no longer comes into direct contact with the blood, but is in contact with a saline solution contained in a rubber finger-stall which projects into the valve chest. Consequently, while the piston continues to be automatically lubricated, there is no risk of any oil contaminating the blood, and there is no blood side-tracked in the pump. If, by any chance, the finger-stall should split during an experiment, the pump reverts to its original condition and the experiment need not be stopped.

(2) In order to avoid any risk of contaminating the blood from the outside of the lungs and heart (by pericardial fat, etc.), a cannula was tied into the auricular-ventricular groove, and the blood issuing from the pulmonary veins led off to the reservoir.

These modifications were made after histological examination of the earlier experiments, which showed fat or paraffin emboli in the kidneys. Bacterial emboli were also seen to be present in some of the early experiments, and the whole apparatus was consequently washed out with alcohol, followed by sterile saline immediately before use. This procedure was successful in eliminating them. The apparatus was in duplicate, and so arranged that two kidneys (ordinarily from the same animal) could be perfused either from a common source of blood, or from two entirely different circuits.

Protein in the urine and in the solutions added to the circulating blood, was estimated by the method of Kerridge [1931]. Care was taken that the quantity of protein added was never less than that necessary to make such a concentration in the blood that, if it were excreted in this concentration in the urine, it would be easily estimated by this method (about 25 mg. protein nitrogen per 100 c.c.). Smaller concentrations (5–25 mg./100 c.c.) were easily detectable. In the anæsthetized animals the protein, dissolved in 0·9 p.c. NaCl, was slowly added through the cannula in the jugular vein. In the perfusion experiments the protein in saline solution was either added initially to the circulating blood, or at a given time in the course of the experiment after a control period. Particulars concerning individual proteins are given together with the results.

The *Creatinine* excretion was followed in most experiments with isolated kidneys, creatinine being added to the circulating blood to raise the concentration to about 10 mg./100 c.c. Estimations were made by the method of Folin as described by Rehberg [1926]. From the concentrations of creatinine in the urine and the serum, we have calculated the glomerular filtration in the manner described by Rehberg [1926]. We are not, however, prepared at this stage to affirm that this figure represents the actual rate of passage of water through the glomerular membranes of the isolated kidney, but we have found it a useful index of the activity of the preparation.

As a rule, a sufficient rate of urine flow was obtained without the addition of any diuretic. In some experiments, both on the intact animal and the isolated kidney, it was necessary to add 25–50 c.c. of normal saline. In no case was urea or glucose added. Toxic reactions (marked reduction in urine flow, blood flow and filtration rate) were obtained when certain of the protein solutions were added to the pump-lung-kidney preparation. These, however, were in most cases less than those obtained when similar quantities of fresh defibrinated blood were added.

Histological examinations were made of a certain number of dogs' kidneys, both experimental and control. The latter included some from dogs used merely as "bleeders."

Thin wedges of the kidneys were fixed in 4 p.c. Schering formaldehyde in 0·9 p.c. NaCl, and sections were prepared from paraffin and frozen blocks. The paraffin sections were stained with Ehrlich's hæmatoxylin and eosin, Weigert's iron hæmatoxylin and van Gieson's mixture, and Weigert's fuchselin and neutral red. Sections cut on the freezing microtome were stained for fat with Scharlach R, and counterstained with Ehrlich's hæmatoxylin. In a few examples portions of kidney were also fixed in Zenker's fluid, but as no additional information was obtained by this procedure the use of this fixative was not continued.

Control observations.

The first few samples of urine collected from the anæsthetized cats not infrequently contained protein. This very rapidly cleared up, and was probably due to the excitement during the anæsthesia, and possibly, also, to reflexes from the bladder and urethra during the cannulation.

The urine secreted by the isolated kidneys was free from protein, with the exception of the first few samples collected after transference to the pump-lung-kidney circuit. The first 2 c.c. of urine secreted were rejected as being contaminated by urine secreted before excision, and the second 2 c.c. sample contained usually about 20–100 mg. protein nitrogen per 100 c.c., while the protein excretion rate varied from kidney to kidney from 50 to 200 γ of protein nitrogen per min. This proteinuria usually cleared up almost completely in about $\frac{1}{2}$ hour.

The isolated kidneys usually continued to secrete for some 3 hours after the beginning of perfusion. Towards the end of this period both urine flow and filtration rate was small, probably on account of the approaching death of the kidney, but it is to be noted that in no case was there a concomitant increase in protein excretion. Death of the kidney, therefore, is not accompanied by proteinuria.

The time elapsing between clamping the renal artery preparatory to excision of the kidney and the establishment of the flow of blood from the pump was usually between 1 and 2 min. Control experiments have shown that stopping the perfusion pump during the course of an experiment for as long as 5 min. does not give rise to proteinuria, although anæmia for 30 min. does. There was, moreover, no correlation between the magnitude of the initial protein excretion rate, and the time taken in transferring the kidney. We are of the opinion, therefore, that the initial proteinuria is not due to the anæmia produced by the transfer of the kidney from the whole animal to the pump-lung-kidney circuit, but results, in all probability, from the disturbance to the renal nerves incidental to the operation. We are supported in this conclusion by the observations of Livingston and Waggoner [1925], who found that in

the anæsthetized rabbit, obstruction of the flow of blood through the kidney did not produce proteinuria, if care were taken that the renal nerves were not interfered with in any way.

Histological examination of nine kidneys of dogs used merely as "bleeders" was made, of which five could be regarded as normal kidneys. In these the arrangement and appearance of the renal elements resembled in general those found in the human kidney. In particular the glomeruli presented a marked structural similarity. Sclerotic glomeruli, such as are found in all human kidneys in varying extent in the later decades of life, are, however, not present in the examples in this series. Likewise, the different forms of vascular degeneration that are responsible for these appearances in human kidneys are not present in the dog kidneys. In all examples the capsular space in a few of the glomeruli contained a small amount of a vacuolated hyaline eosinophil material of albuminous appearance.

The cytological differences that exist between the first and second convoluted tubules in the human kidney are not present in the dog's kidney. A further difference lies in the prevalence of large quantities of isotropic fatty material in the high cubical epithelium of Henle's loops. This has been observed so constantly both in this and in the two following series that it must be regarded as a normal feature for the mongrel dogs which were used for these experiments.

In one kidney of this series there was a chronic ascending nephritis which, from the post-mortem examination, appeared to be secondary to a myoadenomatous enlargement of the prostate gland. In another kidney a very few of the glomeruli showed a focal necrosis of the tuft, a lesion that is commonly found in human Bright's disease. In this change there is a focal degeneration of a fatty or lipoid nature with loss of the nuclei in the affected portion of the tuft. In the same kidney there was one small focus of periglomerular infiltration with small lymphocytes. In two other examples in this series, also, were found a few small foci of lymphocytic infiltration and, in some of these areas, there was also a little fibrosis. The precise nature of these changes must remain uncertain, but, inasmuch as in two of these dogs the renal lesion was associated with a granulomatous inflammation of the spleen resembling Hodgkin's lymphogranuloma, it may be inferred that the slight chronic focal inflammation of the kidney was part of the same condition.

Three kidneys were examined which had been used as controls in the isolated perfusion experiments, and the alterations in the histological appearances in the dog's kidneys resulting from perfusion for 3–4 hours

are remarkably slight. The only demonstrable change is a certain amount of œdema of the interstitial tissue. This is most conspicuous about the glomeruli and results in the production of a narrow empty halo immediately around Bowman's capsule (Plate I, fig. 1). The glomerular tufts are plump from distension of their capillaries. There is no indication, from the histological appearances of the cells of either glomeruli or tubules, that the nutrition of the kidney has been impaired by perfusion.

In the earlier specimens there were two kinds of artefact produced in the process of perfusion. In the first, observed in sections stained for fat, a number of the glomerular capillaries contained small fat emboli. In the second, which involved both glomerular capillaries and some of the larger vessels, such as the interlobular arteries, the lumina sometimes contained closely packed masses of cocci without any surrounding cellular reaction. The modifications in the technique of perfusion previously referred to stopped the occurrence of these two kinds of emboli, and in the later specimens they were absent or present to only a very trivial extent.

In one example a small focus of lymphocytic infiltration was present in the interstitial tissue of the cortex.

Results.

The results on the anæsthetized animals, and on the isolated perfused organs, agreed with one another and are summarized in the following table, together with the values of the molecular weights of the proteins obtained from the literature.

	Molecular weight of protein		
	By ultra-filtration method*	By ultra-centrifugation method†	By osmotic pressure method‡§‖
Proteins excreted:			
Gelatin	123,600	35,000	>16,500 <66,700‡
Bence-Jones	24,500	35,000	—
Egg albumin	33,400	34,500	—
Hæmoglobin	66,800	68,000	67,000§
Proteins not excreted:			
Hæmoglobin	66,800	68,000	67,000§
Serum albumin	45,000	67,500	72,000¶ 75,000‖
Serum globulin	81,000	103,800	>154,000¶ <192,000
Edestin	116,000	208,000	—
Casein	192,000	188,000	—
Hæmocyanin (*Helix*)	—	5,000,000	—

* Cohn [1925]. † Svedberg and co-workers [1930, etc.].
‡ Frankel [1931]. § Adair [1928]. ‖ Burk [1931].
¶ Adair and Robinson [1930].

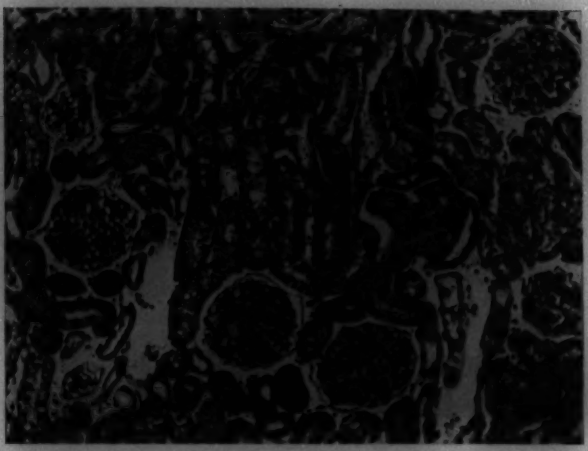

Fig. 1. Section through a dog's kidney which had been isolated and perfused for 1¼ hours with normal defibrinated blood. Stained with hæmatoxylin and eosin. Magnification × 140.

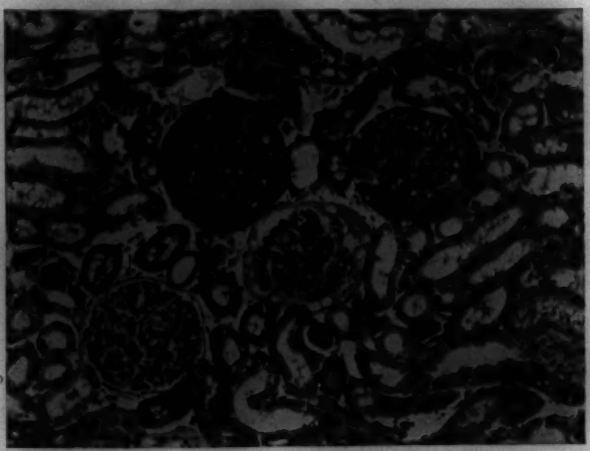

Fig. 2. Section through a dog's kidney which had been isolated and perfused with normal dog's blood for 1¼ hours and with blood containing egg albumin at a concentration of approximately 50 mg. protein nitrogen per 100 c.c. blood for ½ hour. Magnification × 140.

Fig. 3 illustrates an experiment on the perfused dog's kidney in which dog's blood containing gelatin was alternately substituted for normal dog's blood without stopping the circulation. In Fig. 4 are plotted the results of an experiment in which egg albumin was added to the blood perfusing a dog's kidney. Fig. 5 is drawn from data obtained from two anæsthetized cats, showing that Bence-Jones protein was excreted, while hæmocyanin was not. The diuretic effect seen in both cases is explained by the 0·9 p.c. NaCl solution in which the protein was dissolved.

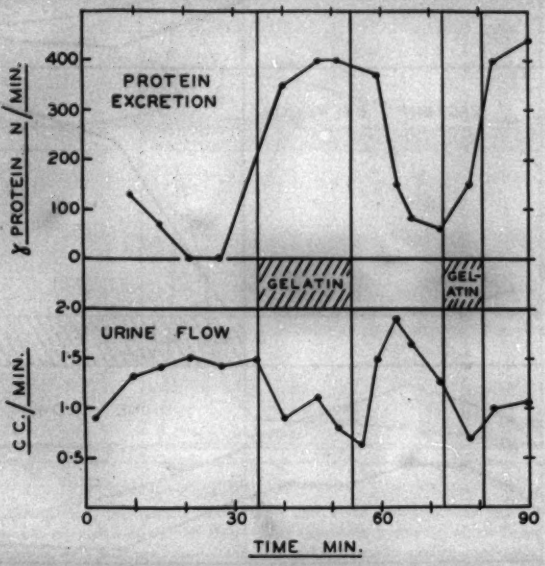

Fig. 3. Pump-lung-kidney preparation. During the period shown, the kidney was perfused with blood containing gelatin (approximately 150 mg. protein nitrogen per 100 c.c.). The resulting proteinuria is seen to be reversible.

Details of the preparation and excretion of the individual proteins are given below.

(1) *Gelatin.* Pickford and Verney[1] have found that gelatin can be excreted by the heart-lung-kidney preparation. Its molecular weight is a little doubtful. The minimum molecular weight, calculated from

[1] Personal communication.

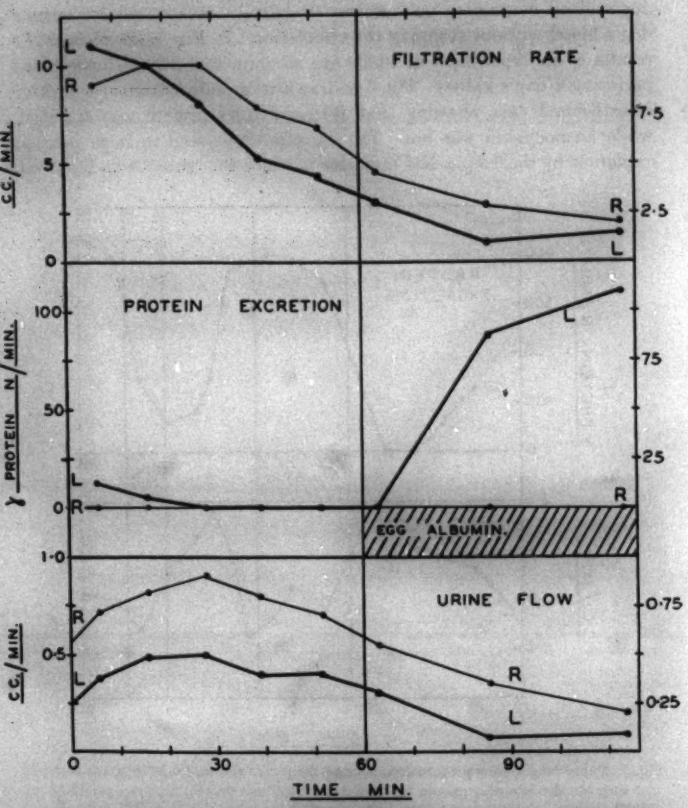

Fig. 4. Pump-lung-kidney preparation. The right kidney was perfused with normal defibrinated blood throughout (thin lines). Egg albumin was added to the blood perfusing the left kidney to make a concentration of about 150 mg. protein nitrogen per 100 c.c. at the moment indicated (thick lines). The filtration rate was calculated from the creatinine excretion.

chemical analyses, is about 10,300 [Cohn, Hendry and Prentiss, 1925], and Krishnamurti and Svedberg [1930], approaching the problem by the method of ultra centrifugation, find that ordinary gelatin solutions are heterogeneous at all H-ion concentrations. At a *p*H of 7·5 the greater part of the gelatin appears to have a molecular weight of 35,000, but in the vicinity of the iso-electric point (*p*H 4–6), aggregation takes place, and

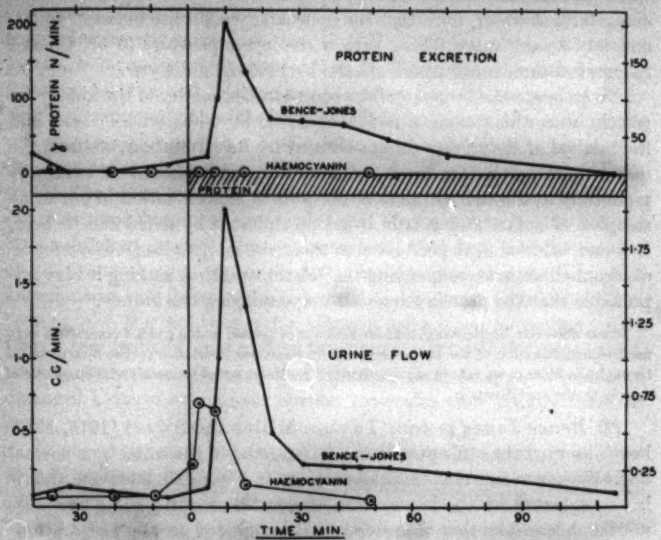

Fig. 5. Composite figure drawn from results obtained on two cats under dial anæsthesia. Into one was injected a solution of Bence-Jones protein containing about 10 mg. protein nitrogen, and into the other a solution of hæmocyanin (*Helix*) containing 160 mg. protein nitrogen. The times of injection are made to correspond in the two experiments. The Bence-Jones protein was excreted, the hæmocyanin was not.

different preparations may have different molecular weights ranging from 75,000 to 100,000. At all H-ion concentrations, components with a molecular weight of 11,000 were present in addition: Krishnamurti and Svedberg suggest that these may be breakdown products of the true gelatin molecules whose molecular weight is 35,000. Cohn [1925], from a study of the diffusibility of gelatin through collodion membranes, comes to the conclusion that the molecular weight is not less than

123,600. He does not state the H-ion concentration of his solutions, but if, as is very probable, he worked at the iso-electric point, his results do not conflict with those of Krishnamurti and Svedberg. In the circulating blood it seems highly probable that gelatin has a molecular weight of 35,000, there being present, also, a certain proportion of breakdown products with a molecular weight of about 11,000. Frankel [1931], from measurements on the osmotic pressure of solutions of pure commercial gelatin in water, finds that the molecular weight lies between 16,500 and 66,700, the value falling with a rise in temperature (6–38° C.) and rising after removal of traces of salts by prolonged dialysis.

No estimate can be given of the concentrating power of the kidney for gelatin, since this protein is not precipitated by tri-chloracetic acid, and the method of Kerridge cannot be used for its estimation; we used the quantity of precipitate produced by a standard amount of saturated picric acid as a rough estimate of the protein concentration of our urine samples. The fact that gelatin is not precipitated by trichloracetic acid, however, enabled us to perceive that there was no simultaneous excretion of serum albumin accompanying the gelatin, and thus making it very improbable that the protein excretion was damaging the kidney.

It was observed, incidentally, that the addition of gelatin to dog's blood results in a very marked agglutination of the corpuscles, and an increased resistance to the flow of blood through the kidney; we cannot say whether this indicates a real vaso-constriction, or is due merely to the agglutination.

(2) *Bence-Jones protein.* Taylor, Miller and Sweet [1916, 1917] have observed that this protein can be excreted in the urine by a normal dog. We have confirmed this observation, and found, in addition, that it is concentrated by the kidney, the concentration ratio being probably not much less than that of creatinine, although our protein preparations were not pure enough for the exact figure to be determined. We are indebted to Dr L. J. Hewitt for our samples which were prepared from the urine of patients at the London Hospital.

(3) *Egg albumin.* We made no attempt to prepare a pure sample of egg albumin, but merely diluted the white of an egg with about 50 c.c. of normal saline solution and filtered it through muslin. It is stated by Claude Bernard [1859] that Berzelius noticed that egg albumin could be excreted by the kidney, and this observation has since been repeatedly confirmed. Our results are a further confirmation, and we have found that the urine contains the protein in a higher concentration than does the circulating blood, the concentration ratio being approximately the same as that of creatinine.

(4) *Hæmoglobin.* It is well known that hæmoglobin can be excreted by the kidney, but it was observed by Pearce, Austin and Eisenbrey [1912] that hæmoglobin is only excreted by the intact dog when its concentration in the blood exceeds a certain threshold value. We have been able to confirm this, and have therefore included it in both sections of the table. On the isolated kidney, the threshold lies between 0·2 and 0·5 g. Hb per 100 c.c. serum. Even when this threshold is exceeded, however, hæmoglobin is not very readily excreted, the concentration ratio being always very much less than that of creatinine, although running parallel to it, and is rarely greater than 1. The threshold value given by Pearce, Austin and Eisenbrey is 0·06 g. hæmoglobin per kg. body weight. Assuming $\frac{1}{15}$th of the body weight to be plasma, this corresponds to a concentration of 0·15 g. hæmoglobin per 100 c.c. plasma, which is of the same order as the figure obtained by us.

(5) *The serum proteins.* The normal kidney appears never to excrete either serum albumin or serum globulin, even though they be derived from a different species. Thus we have shown that dogs' isolated kidneys do not excrete human, cat or ox serum proteins, and that the kidneys of anæsthetized cats do not excrete human or dog serum proteins. In conformity with the fact that serum albumin has a lower molecular weight than serum globulin, it is well known that in nephritis the urinary proteins contain more albumin than globulin. Thus it appears that even damaged kidneys excrete the smaller molecules more readily than the larger.

In one experiment we perfused two kidneys with a mixture of about 30 p.c. ox blood to 70 p.c. dogs' blood. The ox blood was somewhat hæmolysed, and hæmoglobin appeared in the urine. The serum proteins of the ox were not excreted at all, even though their concentration in the blood plasma was about the same as that of the hæmoglobin. We are therefore convinced that the same kidney can excrete hæmoglobin, and hold back "foreign" serum proteins. This conclusion is supported by the observations of Cramer [1908], who states that ox serum proteins injected intraperitoneally into normal rabbits are not excreted in the urine, whereas egg albumin, similarly injected, is excreted.

The evidence concerning the relative molecular weights of hæmoglobin and serum albumin is conflicting. Cohn [1925] (ultrafiltration method) considers hæmoglobin to be the larger, Svedberg [1930] (ultracentrifugation method) considers them to be the same, while Adair and Robinson [1930] and Burk [1931] (osmotic pressure measurements) consider hæmoglobin to be the smaller.

(6) *Edestin.* We do not feel much confidence in the results obtained with this protein, owing to its low solubility. It was necessary to dissolve the requisite quantity of the protein (obtained from the British Drug Houses, Ltd.) in 4 p.c. sodium chloride at a *p*H of 8–9, and to add it to the blood very slowly, so that it did not precipitate out. Moreover, Svedberg and Stamm [1929] have shown that in concentrations corresponding to saturation in the blood serum, edestin is unstable, and tends to break down into products of low molecular weight, not identifiable by the ultracentrifuge. In conformity with this, we observed a small proteinuria in one cat, after the intravenous injection of 10 c.c. of a 2·4 p.c. solution of edestin in 4 p.c. NaCl at a *p*H of 8·5.

(7) *Casein.* We used "Light White Casein" from the British Drug Houses, Ltd. The molecular weight of casein appears to depend upon the manner in which it is prepared. Thus, Svedberg, Carpenter and Carpenter [1930] obtained (1) a preparation consisting of a mixture of substances with molecular weights ranging from 75,000 to 100,000, (2) a preparation consisting almost entirely of a substance with a molecular weight of 188,000, with a small admixture of a substance with a molecular weight of 375,000, and (3) a preparation containing only the substance of molecular weight 375,000. While it seems fairly probable that in the circulating blood most of the casein would have a molecular weight of 188,000, the matter is not of great significance, since the lowest molecular weight given is greater than 68,000, which appears to be the excretion limit for the normal kidney.

(8) *Hæmocyanin.* No attempt was made to obtain a pure preparation of hæmocyanin. About thirty *Helix pomatia*[1] were bled by careful incision of the heart, the mixed blood centrifuged to remove sediment, and dialysed in the cold for 6 days against 0·9 p.c. NaCl. The final solution contained 450 mg. protein nitrogen per 100 c.c.

Histological preparations were made from eight kidneys which were excreting protein, and in general the appearances could not be distinguished from those of the control perfused kidneys. In one kidney, however, which was excreting egg albumin in high concentration at the time of fixation, there is a conspicuous ballooning of most of the glomerular capsules with a dense homogeneous hyaline eosinophil substance (Plate I, Fig. 2). This substance is denser and more eosinophil than the scantier material found in the capsular spaces in the control kidneys. The lumina of the tubules are also filled with similar material. In another

[1] We are indebted to Mr N. H. Howes for these snails and for his assistance in preparing the hæmocyanin solutions.

kidney, which was also excreting egg albumin, but in a lower concentration, the amount of "albuminous" material in the capsules is in excess of that usually present.

Conclusions.

Our results show that while the mammalian kidney can excrete some proteins, it cannot excrete others. The distinction does not appear to depend on chemical structure, or biological origin, but on molecular weight. Proteins with molecular weights less than about 70,000 are excreted, those with heavier molecules being retained. The histological preparations showed no evidence of damage in kidneys which were excreting protein. In two experiments, in which large amounts of egg albumin were being excreted, there was positive evidence that the glomeruli were the sites of excretion. This is in accordance with the observations of several workers of 40–50 years ago (for references, see Cushny, 1926).

The filtration theory of glomerular function has postulated that the glomerular membranes are impermeable to all proteins. Cushny [1926] states that "the excretion of injected protein seems to occur only with injury to the capsule." Our chemical and histological evidence does not support either of these postulates, but the observed phenomena can all be easily accounted for by the filtration theory, if it be assumed that the glomerular membrane is permeable not only to crystalloids but also to proteins of molecular weight less than 70,000. Indeed, it is difficult to imagine any other theory which would explain so easily the differentiation of proteins by the kidney according to their molecular weights.

Summary.

The excretion of proteins by anæsthetized cats and rabbits and by the isolated and perfused kidneys of dogs has been studied.

Gelatin, Bence-Jones protein, and egg albumin were excreted and concentrated; serum albumin and globulin of their own and other species, edestin, casein and hæmocyanin were not excreted. Hæmoglobin was excreted only when its plasma concentration exceeded a certain level, and was little concentrated.

The proteins which were excreted all have molecular weights less than 70,000, while those which were not excreted all have molecular weights greater than 70,000. Hæmoglobin has a molecular weight of 68,000 and is thus near the border-line.

Histological examination showed no evidence of renal damage and protein was seen in the capsular space in two experiments in which egg albumin was excreted in high concentration.

An account has been given of the histological picture of the normal dog kidney, and of the kidney which has been isolated and perfused for 3–4 hours; the difference between them is surprisingly small.

Our results are explained by the filtration theory of glomerular function if it be assumed that the glomerular membrane is permeable not only to crystalloids but also to proteins of a molecular weight less than 70,000.

It is our pleasure to thank Prof. Arthur Ellis and Prof. C. Lovatt Evans for advice and criticism.

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THE PORTAL CIRCULATION.

II. The action of acetylcholine.

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In a previous paper [McMichael, 1932] it was shown that adrenaline constricted the portal venous ramifications within the liver, thus confirming the views of others, *e.g.* François-Franck and Hallion [1896], and Griffith and Emery [1930], that the post-ganglionic splanchnic nerves passing into the liver carry constrictor fibres to the portal venules. In addition, it was shown that when the splanchnic arterioles dilated with the fall in arterial pressure after adrenaline injection, there occurred a secondary rise in portal pressure, due to increased inflow into the portal system. Since recent work by Pick [1931], and by Grab, Janssen and Rein [1929], has tended to stress the importance of the hepatic vascular bed in regulating the flow of blood to the right heart, it is desirable to know whether there is a parasympathetic supply having the converse effect of adrenaline and the sympathetic nerves on the hepatic vascular system. It was thought that acetylcholine might yield a clue to this problem, and the series of experiments detailed in this paper was therefore carried out, as a corollary to the work with adrenaline.

Technique and methods.

The arrangement of the experiments was in most respects identical with that used for the adrenaline investigations [McMichael, 1932]. The work was carried out entirely on cats under chloralose anæsthesia, and the dosage of acetylcholine ranged from 0·0005 to 0·5 mg. for an average cat of 3 kg. The dosage and mode of administration of the drug is indicated in each tracing, or its accompanying legend. The portal and vena cava pressures were recorded by water manometers connected to the stump of the splenic vein and the left renal vein respectively. The liver volume was recorded by a plethysmograph after the model devised by Griffith and Emery [1930]. The flow from the inferior mesenteric vein was measured by a Condon drop recorder. To record changes occurring in the stroke

volume of the heart, a Henderson glass cardiometer was used, connected to a tambour with a slack rubber membrane. As it proved difficult and also unnecessary for the present purposes, to take records showing the diastolic heart volume, a side valve was provided on the connecting tube. In this way diastolic volume is not indicated in the cardiometric tracings, which only show the relative magnitude of the rapid change in volume which occurs in systole. In the cat it was found difficult to take more than three records of the type mentioned at any one time, but as the general nature of the changes did not vary, it is possible to piece the observations together into a composite picture, with some degree of certainty as to their value in relation to one another.

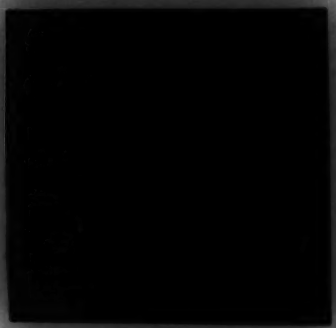

Fig. 1.

Fig. 2.

Fig. 1. Upper record: portal venous pressure. Middle record: carotid pressure. Lower record: vena cava pressure recorded from renal vein. Time record: 10 sec. At the first arrow 0·2 mg. acetylcholine was injected into the saphenous vein. The second series of three arrows indicates corresponding times about 6 sec. after the arterial pressure has begun to fall. At this time the portal venous pressure begins to rise, while little significant change occurs in the vena cava pressure.

Fig. 2. Upper record: portal venous pressure. Middle record: arterial pressure. Lower record: vena cava pressure from renal vein. Time record: 10 sec. At the time marked by the arrows 0·2 mg. acetylcholine was injected intraportally. There is an immediate rise in portal pressure followed in a few seconds by a fall in arterial pressure. No significant change is seen in vena cava pressure.

I. The effect of large doses of acetylcholine.

The injection of 0·2–0·5 mg. acetylcholine into a systemic vein produces, as is well known, a marked fall in blood-pressure, together with slowing of the heart. The portal pressure, after showing no change for

some 5 or 6 seconds following the commencement of the drop in arterial pressure, then begins to rise (Fig. 1). This rise in portal pressure is gradual during the first few seconds, and then it climbs more steeply, ultimately falling away as the arterial pressure returns to normal. The injection of a similar dose, or even of a somewhat smaller dose, directly into a mesenteric vein, invariably produces a steep rise in portal pressure, which occurs before any change is seen in the arterial pressure (Figs. 2, 3). It is probable therefore that the rise in portal pressure, caused by the

Fig. 3. Upper record: portal pressure. Lower record: carotid pressure. Time record: 10 sec. The intraportal injection of small doses is seen to bring about a rise in portal pressure, which results from a direct constricting effect on the vein.

injection of large doses of acetylcholine into systemic veins, is due to direct action on the portal vein, producing either constriction of the portal venous ramifications in the liver, or diminution in the calibre of the main trunk of the vein itself. Since there is no change in liver volume immediately after intraportal injection of the drug (Fig. 6), it is likely that the action is a general one on the musculature of the portal venous system. A somewhat similar effect was noted by Fleisch [1931], who observed that large doses of acetylcholine caused constriction of the mesenteric veins. Since this action is probably exerted directly on plain muscle, and is not related to the parasympatho-mimetic action of the drug, its nature will not concern us further in this investigation.

II. The effect of small doses of acetylcholine.

(a) *The portal pressure changes.*

Doses of acetylcholine under one-fifth of a milligramme injected into a systemic vein produce a succession of changes in the portal pressure different from those described above. The first change takes place in the arterial pressure (Fig. 4). This falls away steeply, while the portal pressure

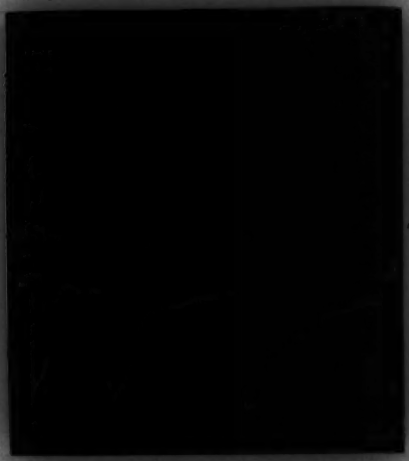

Fig. 4. Upper record: portal pressure. Lower record: arterial pressure. Time record: 10 sec. Three successive records from the same animal showing the effect of different small doses of acetylcholine into the saphenous vein. Vertical lines mark corresponding times. 0·001 mg. brings about a fall in portal pressure with a scarcely significant secondary rise. With increasing doses (0·01 and 0·02 mg.) the secondary rises become more marked.

remains unchanged for some 6 seconds before it also begins to fall. As the cardiac slowing passes off it is followed by an acceleration of the heart beat, above the normal, for a few seconds. About this time the fall in portal pressure ceases, and is replaced by a rise. The extent of this rise is dependent upon the dosage of acetylcholine and the degree of the initial fall in blood-pressure. With a dose of 0·02 mg. the blood-pressure has fallen very considerably and the subsequent rise in portal pressure is

correspondingly well marked. With smaller doses of acetylcholine, the rise in portal pressure becomes less prominent.

Excluding general effects on the calibre of the portal vein as a whole, the cause of a fall in portal pressure may be either increased outflow or diminished inflow. In view of the work of Dale [1914], and the general acceptance of the hypothesis that acetylcholine dilates arterioles, thus causing an increased flow through these vessels into the veins, it was thought that the most probable cause of the initial fall in portal pressure would be increased outflow from the portal venous system. To investigate this possibility records were taken of the effect of these small doses of acetylcholine on the liver volume, and on the pressure in the vena cava.

Fig. 5. Upper record: portal pressure. Middle record: carotid pressure. Lower record: vena cava pressure. Time record: 10 sec. Note the abscissae marked by arrows on the right of each tracing. (*a*) 0·02 mg. acetylcholine into the saphenous vein. The portal pressure begins to fall 5 or 6 sec. after the arterial fall, and simultaneously (arrows) the vena cava pressure undergoes a very slight rise. (*b*) 0·05 mg. acetylcholine into the saphenous vein. The fall of arterial pressure is accompanied by marked cardiac slowing. Simultaneously (arrows) with the fall in portal pressure a distinct rise in vena cava pressure is seen.

(*b*) *The effect of acetylcholine on liver volume and vena cava pressure.* (See also Section (*g*).)

With doses of acetylcholine ranging from 0·001 to 0·2 mg. the effect is constantly a diminution of liver volume (first part of Fig. 15). This diminution in the size of the liver begins about 6 seconds after the arterial

pressure has begun to fall, *i.e.* at the same time as the fall in portal pressure. Fig. 5 shows simultaneous tracings of arterial pressure, portal pressure and vena cava pressure. It is seen that, with smaller doses of acetylcholine (0·02 mg.), little change occurs in the pressure in the vena cava. Larger doses, however, cause a rise in vena cava pressure which occurs at the same time as the fall in portal pressure, and presumably also simultaneously with the diminution in liver volume. This rise in vena

Fig. 6. Upper record: liver volume. Lower record: carotid pressure. Time record: 10 sec. Successive doses of 0·01, 0·02 and 0·03 mg. acetylcholine were injected intraportally. It is seen that no change occurs in liver volume until the fall in arterial pressure appears The diminution in liver volume is proportional to the degree of the fall in blood-pressure.

cava pressure is only present when cardiac slowing is marked. It was thought at first that this series of changes might be interpreted by assuming that acetylcholine exerted a dilator action on the hepatic veins, thus causing an increased outflow from the liver lobules, with a consequent fall in the liver volume and portal pressure, and an increased inflow into the vena cava. This increased inflow into the vena cava would manifest itself as a rise in vena cava pressure, only in the presence of cardiac slowing: otherwise the heart would be able to deal with the increased delivery of blood quite efficiently, and the vena cava pressure would remain un-

altered. Experiments carried out to test this hypothesis, however, showed that it was incorrect.

The invalidity of the assumption was at once proved by the experiment illustrated in Fig. 6. If acetylcholine dilated the hepatic veins, we should be able to demonstrate a fall in liver volume by the injection of a suitable dose into the portal system, and this diminution in volume should precede any effect on the general systemic circulation. As the illustration shows, this postulate is not fulfilled. No alteration in liver volume occurs until the arterial pressure has fallen, and further, the diminution in liver volume appears to be dependent upon the extent of the fall in blood-pressure.

The true explanation of the initial fall in portal pressure, and of the concomitant diminution of liver volume, is therefore to be sought in the changes which occur in inflow rather than in alterations of the outflow from the portal system.

(c) *The flow of blood through the splanchnic arterioles under the influence of acetylcholine.*

Fig. 7 shows the type of result constantly obtained when the flow from a mesenteric vein is measured under the influence of acetylcholine. During the first 6 seconds after the arterial pressure begins to fall, there is little change in the rate of flow. A distinct slowing then makes its appearance, and becomes most marked about the turning-point of the arterial pressure curve. As the pressure rises the slowing continues to be pronounced, and as the pressure reaches a level approaching its original height the rate of flow again returns almost to its original rate. Thus the initial fall in portal pressure, which occurs with acetylcholine, appears to be dependent on diminished inflow into the portal system through the splanchnic arterioles.

This result was at first surprising, in view of the opinions generally held as to the action of acetylcholine. It must be remembered, however, that the observations from which conclusions were reached regarding the action of the drug on the arterioles, were not as a rule made on the intact animal, but on isolated perfused organs and tissues. This finding does not therefore in any way invalidate the classical work on the vaso-dilator action of the "vagus substance." It is of interest in passing to note the difficulty which Reid Hunt [1914] found in accepting the work of Dale [1914]. The former writer was unable to detect vaso-dilatation with acetylcholine, and found himself forced to explain the fall in blood-pressure by diminished cardiac output.

Accepting the view, which has been amply proved by other workers, that acetylcholine dilates arterioles, we have two possible explanations for the diminished inflow into the portal system in the intact animal. The first is, that the vaso-dilatation may be more marked in the limb vessels, and the second, that the cardiac output may be diminished during the first stage of acetylcholine action.

Fig. 7. Upper record: carotid pressure. Lower record: flow from a mesenteric vein in drops. Time record: 2 sec. Average rates are given in each marked interval. At the arrow 0·002 mg. acetylcholine was injected into the saphenous vein. During the first 6 sec. there is little change in the rate of flow. A distinct slowing then makes its appearance. When the arterial pressure begins to rise the slowing becomes still more marked, and the flow only begins to increase again when the blood-pressure approaches the normal level.

(*d*) *The flow of blood through the limb vessels under acetylcholine.*

Fig. 8 shows the flow of blood from the femoral vein under the influence of acetylcholine. During the stage of cardiac slowing the flow is somewhat diminished. As soon as this stage has passed off, however, a distinct acceleration of flow above the normal level is seen, which gives way to a slowing as the pressure begins to rise again. It appears then that the cardiac output must be diminished during the stage of cardiac slowing, since there is no acceleration of the flow either in the splanchnic area or

in the limbs. The subsequent acceleration which occurs in the flow from the femoral vein indicates, however, the persistence of vaso-dilatation in the limbs until the pulse rate has begun to recover. Even in the intact animal therefore, we find evidence of vaso-dilatation in the limbs, although this is masked by the diminution in cardiac output during the stage of cardiac slowing. In the intact animal there is no such evidence of vaso-dilatation in the splanchnic area, so that if such exists, it must be less marked than that which we have found in the extremities.

Fig. 8. Upper record: carotid pressure. Lower record: flow from femoral vein in drops. Time record: 2 sec. Following the injection of 0·02 mg. acetylcholine into the jugular vein the blood-pressure falls, and marked cardiac slowing occurs. During the stage of cardiac slowing, there is a diminution of the flow from the femoral vein. When the bradycardia passes off the flow is increased but is later diminished during the recovery of the blood-pressure.

(*e*) *The cardiac output under acetylcholine.*

The output of the heart under this drug may be influenced in various ways. The slowing of the heart itself may diminish the minute volume, although the stroke volume may be increased. Secondly we have the effect of peripheral vaso-dilatation in increasing the capacity of the vascular system, so that less blood returns to the heart, and its output is diminished.

Regarding the first possibility there can be no reasonable doubt. We have mentioned the evidence of diminished cardiac output in discussing the changes in flow through the limbs during the stage of cardiac slowing. There is also evidence of it in the rise in vena cava pressure, which has

already been noted to occur only when the cardiac slowing is marked (Fig. 5). With the vagi cut to accentuate cardiac slowing from acetyl-

Fig. 9. Upper record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the first arrow 0·2 mg. acetylcholine was injected. With the fall in blood-pressure, the heart is slowed from 258 to 120 per min., and the amplitude of each beat is increased. For a few seconds the heart beat becomes irregular, and at this stage the output per beat is markedly diminished. The blood-pressure then begins to recover, and the output per beat gradually returns to normal.

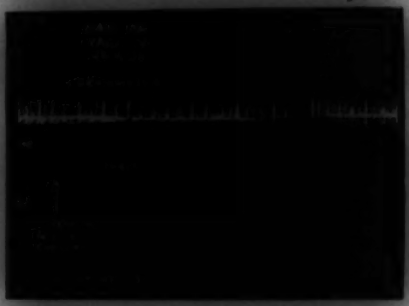

Fig. 10. Upper record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the arrow 0·0005 mg. acetylcholine was injected. This dosage is insufficient to produce cardiac slowing. As the blood-pressure falls the cardiac output becomes diminished, and increases again as the pressure rises.

choline, the cardiometer shows an increased output per beat, which, however, is insufficient to maintain the normal minute volume (Fig. 9).

The second factor, *i.e.* increased capacity of the vascular system, is one

which plays a special part in the failing preparation in which there is a condition of oligæmia. The volume of the circulating blood is diminished, and vaso-dilatation in the periphery further diminishes the return of blood to the right heart, with resultant diminution in cardiac output independent of slowing (Fig. 10). In the failing preparation the effect of acetylcholine on the portal circulation is modified, and the fall in portal pressure becomes the predominant feature, while the secondary

Fig. 11. Upper record: portal venous pressure. Middle record: cardiometer. Lower record: carotid pressure. Time record: 5 sec. At the arrow, 0·01 mg. acetylcholine was injected into the saphenous vein. The heart rate is slowed from 240 to 180, and as the slowing passes off, a diminution in output becomes evident. The fall in portal pressure corresponds to the stage of cardiac slowing with diminished cardiac output. The experiment was repeated with the injection of 20 c.c. saline into the circulation immediately after the acetylcholine injection. The cardiac output was raised with a corresponding rise of portal pressure.

rise is usually absent (Fig. 11). The reason for this will be discussed later (p. 418). Under these conditions the portal pressure fall is seen to follow closely the diminution in cardiac output, and the portal fall is abolished when the cardiac output is artificially maintained.

Thus we see that the fall in portal pressure which occurs after the injection of acetylcholine, is dependent on two factors: a predominance of vaso-dilatation in the limbs deflecting blood away from the splanchnic area, and diminished cardiac output.

Fig. 12. Upper record: carotid pressure. Lower record: portal pressure. Time record: 5 sec. Baylisa compensator attached to the femoral artery. Acetylcholine (0·005 mg.) was injected into the saphenous vein. The usual fall in portal pressure is not evident, but after a delay a secondary rise is seen. With the compensator in the circuit, a marked rise in portal pressure begins immediately the carotid pressure starts to fall.

Fig. 13. Upper record: portal venous pressure. Lower record: arterial (femoral) pressure. Time record: 5 sec. Baylisa compensator attached to carotid artery. Following the injection of 0·01 mg. acetylcholine a fall in arterial pressure is accompanied by a fall in portal pressure. With the compensator in communication with the circulation, a rise of portal pressure is seen as soon as the arterial pressure begins to fall, with the same dose of acetylcholine.

(f) The state of the splanchnic arterioles under acetylcholine.

Since we have detected no increase in the flow through the mesenteric arterioles, and since the portal pressure falls and the liver volume diminishes under acetylcholine, the assumption that the splanchnic vessels are dilated would be mere surmise. With a diminished flow through the splanchnic vessels in the presence of diminished cardiac output,

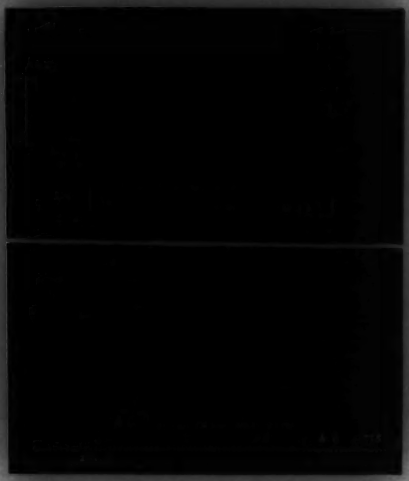

Fig. 14. In both tracings: Upper record: carotid pressure. Lower record: flow from a mesenteric vein (in drops). Time record: 2 sec. Compensator attached to femoral artery. In the upper record 0·01 mg. acetylcholine injected into the saphenous vein brings about a fall in blood pressure with a concomitant diminution of flow through the mesenteric vessels. A few minutes later, with the compensator in communication (lower record), a similar dose of acetylcholine is followed by an immediate acceleration of the flow, with slowing later.

these vessels may be dilated, contracted, or unaltered in calibre. If we could maintain the amount of blood in the arterial system at a constant level, vaso-dilatation in the splanchnic area, if present, should be demonstrable. One way in which it would come to light, would be by an immediate rise in portal pressure as soon as the arterial effect of acetylcholine had manifested itself. These conditions can be achieved by the use of the Bayliss [1908] compensator. Fig. 12 shows the effects of acetyl-

choline under these conditions. There is a prolonged delay, instead of a slight fall, in the first record of portal pressure under acetylcholine, and then the secondary rise is seen as usual. With the compensator in communication with the circulation, the effect is an immediate and pronounced rise in portal pressure at the moment when the arterial pressure begins to fall. The compensator was attached to the femoral artery, and

Fig. 15. Upper record: liver volume. Lower record: carotid pressure. Time record: 5 sec. Compensator attached to the femoral artery. 0·005 mg. acetylcholine into the saphenous vein gives a diminution of the liver volume beginning about 6–8 sec. after the fall in arterial pressure. The compensator is then connected to the circulation and, instead of diminishing, the liver increases in volume along with the fall in arterial pressure.

under these conditions tracings may not show the initial fall in portal pressure as one hind limb is out of the circulation, and its function in deflecting blood from the splanchnic area is in abeyance. When, however, the compensator is attached to the carotid artery (Fig. 13), the initial fall in portal pressure is still seen. With the compensator in communication with the circulation, the fall in portal pressure is replaced by an immediate rise, beginning as soon as arteriolar dilatation is evidenced by the drop in arterial pressure.

Fig. 14 shows the effect of compensation on the flow through the mesenteric arterioles. The upper tracing shows the usual slowing of the

flow as already described (Section (*c*)). With the compensator communicating with the femoral artery, the initial slowing is converted into an increased rate of flow. There is no doubt therefore that the splanchnic arterioles are dilated by acetylcholine, and, when the arterial system is kept filled artificially, that they can transmit an increased amount of blood to the portal system thus causing a rise in portal pressure.

The diminution in liver volume which was described in Section (*b*) is due to diminution in the flow through the splanchnic arterioles. As in the reversal of the effects of acetylcholine on portal pressure and on flow from the mesenteric veins, the compensator also reverses this action on liver volume. Instead of undergoing a shrinkage the liver is seen to swell (Fig. 15).

Fig. 16. Upper record: vena cava pressure. Lower record: carotid pressure. Time record: 5 sec. With the injection of 0·01 mg. acetylcholine a fall of vena cava pressure occurs. The arterial pressure does not recover in the usual manner. The compensator is then put into communication with the circulation, and a restoration of blood-pressure with a rise of vena cava pressure results. 0·01 mg. acetylcholine then causes a sudden and steeper rise in vena cava pressure, which ultimately returns to a normal level with the recovery of blood-pressure. The compensator is then excluded from the circulation and different doses of acetylcholine then produce rises in vena cava pressure which only last as long as the cardiac slowing.

(*g*) *General venous pressure effects.*

In Section (*b*), preliminary mention was made of the effects of acetylcholine on vena cava pressure. A rise in vena cava pressure is only seen in the presence of pronounced cardiac slowing. Slowing of the heart alone,

however, is not sufficient to determine a rise in pressure in the vena cava, as can be seen from the first curve in Fig. 16. In this particular preparation, the volume of the circulating blood had to be increased, before the rise in vena cava pressure became evident. Cardiac slowing then determined the extent and duration of the rise. With a slight degree of slowing, the rise of vena cava pressure was slight, and with more marked bradycardia the rise became correspondingly higher and more prolonged.

Fig. 17. Upper record: vena cava pressure. Lower record: carotid pressure: Time record: 5 sec. With the compensator in communication with the circulation 0·1 mg. acetylcholine was injected into the saphenous vein. During the stage of cardiac slowing, a pronounced rise of vena cava pressure is seen. When the compensator is excluded from the circulation this rise of vena cava pressure does not occur.

When the blood-pressure falls with acetylcholine, the first effect of the compensator is to pour more fluid into the circulation. If cardiac slowing is present this excess of fluid brings about a rise of vena cava pressure. When the slowing passes off and the heart in consequence becomes more efficient, the excess of fluid is removed from the venous side of the circulation and the venous pressure falls (Fig. 17).

It is unlikely that these general venous pressure effects could take place without some influence on the portal pressure. If we take the initial

portal pressure fall and correlate it with the changes of inflow which occur at the same time, we find that the inflow is reduced on an average (four experiments) from 136 to 99 drops per minute. The volume flow through the portal vein must be diminished in the same proportion. The initial portal pressure fall is about 20 mm. water as a rule, *i.e.* from 80 mm. down to 60 mm. water. By Poiseuille's law, volume flow through narrow vessels

$$V = \frac{\pi r^4}{8\eta} p,$$

where r is the radius of the blood vessel, η the coefficient of viscosity of the fluid (blood), and p the pressure. With small doses of acetylcholine we

Fig. 18. Upper record: carotid pressure. Lower record: portal pressure. (Tracings subsequently cross.) Time record: 5 sec. Plethora had been induced by means of the injection of gum saline. Acetylcholine then brings about a rise of portal pressure in place of the usual fall.

have not found any evidence of change in calibre (r) of the portal venules within the liver, and the viscosity of the blood (η) presumably remains unaltered, so that

$$V = pK, \text{ or } V \propto p.$$

A diminution of the volume flow from 136 to 99 would therefore account for a drop in portal pressure from 80 to 58 mm. water. This approximates nearly to the fall of 20 mm. water which was observed in the experiments.

With the compensator communicating with the circulation (Figs. 12,

13), the initial rise in portal pressure may amount to 70–100 mm. water, and yet the only increase of inflow that could be detected was from 64 to 75 drops per minute (Fig. 14). This would account for a rise of portal pressure of only 15 mm. water. It would appear probable that the rise in general venous pressure produced by the use of the compensator with acetylcholine (Fig. 17) is responsible for the remainder of the rise in portal pressure.

From the same argument, part of the increase in liver volume brought about by the compensator is probably due to the rise of pressure in the vena cava.

As shown in Fig. 16, when the vascular system has been overfilled following the use of the compensator, acetylcholine may continue to cause an initial rise in vena cava pressure: in the same way a rise in portal pressure may also be brought about (Fig. 18). This rise takes the place of the usual fall in portal pressure and is followed by a secondary rise.

It is thus seen that acetylcholine in the presence of plethora of the circulation, may bring about a rise in general venous pressure in which the portal system shares. The type of curve then produced (Fig. 17) is mentioned particularly, as it was occasionally seen in cats (2 out of 70) where there had been no artificial overfilling of the vascular system. In three experiments carried out in dogs, this type of portal pressure response to acetylcholine was present in all.

(*h*) *The secondary rise in portal pressure.*

In all animals where the circulatory conditions were good and the blood-pressure well maintained, a well-marked secondary rise in portal pressure was seen following the injection of acetylcholine. This rise begins when the arterial pressure is beginning to recover and, generally speaking, the deeper the trough of arterial pressure and the steeper the subsequent rise, the more pronounced the secondary rise in portal pressure (Fig. 4).

The fact, seen from the records of outflow from the mesenteric vein (Fig. 7), that the portal pressure is rising when the inflow is markedly diminished, renders it obvious that the secondary rise in portal pressure must take place by a diminution of outflow from the portal system. It has also been noted that the cardiac slowing, brought about by acetylcholine, is often followed by a period of increased pulse-rate, above the original level. These observations led to the thought that the sympathetic might play a part in the recovery of blood-pressure following its depression by acetylcholine. This hypothesis was put to the test by the use of ergotamine

and ergotoxine to paralyse the sympathetic. Fig. 19 shows the result of this procedure.

It is seen that when the sympathetic is paralysed, the heart beats more slowly as a result of some loss of sympathetic tone. The injection of adrenaline into the portal system no longer leads to the immediate rise in portal pressure which would result with an intact sympathetic system. No significant change is seen in the portal pressure (? no sympathetic

Fig. 19. Upper record: portal pressure. Lower record: carotid pressure. Time record: 10 sec. Following the administration of 20 mg. ergotoxine, 1 c.c. 1/20,000 adrenaline intraportally causes little significant change in the portal pressure, and, a few moments later, a falling carotid pressure results. Acetylcholine then brings about a fall in carotid pressure with gradual recovery and a fall in portal pressure with no secondary rise.

dilator fibres), and the action of adrenaline on the arterial system is to bring about a fall in pressure. The injection of acetylcholine into the animal then brings about the usual fall in arterial pressure, accompanied by a fall in portal pressure analogous to the initial fall in the intact animal. The recovery from the depression of the arterial pressure is slow and gradual, while the secondary rise in portal pressure is absent. The conditions in this animal, and in that illustrated in Fig. 20, are such that a secondary rise of portal pressure might have been anticipated.

In the previous paper [McMichael, 1932], it was pointed out that the splanchnic vessels in the failing preparation were in a condition of greatly

increased tone, which accounted for the absence of any further contraction with adrenaline. In particular, the adrenaline rise in portal pressure normally resulting from the constriction of the portal ramifications within the liver was absent. It is probable that the absence of the secondary rise of portal pressure in the animal with a failing circulation is due to the same circumstance. (See Fig. 11.)

Fig. 20. Upper record: portal pressure. Lower record: carotid pressure. Time record: 5 sec. After 20 mg. ergotoxine the reaction to adrenaline had been reversed. 0·05 mg. acetylcholine brought about a fall in arterial pressure followed by a fall in portal pressure. The recovery of the arterial pressure takes place at a uniform slow rate and there is no secondary rise in portal pressure.

The evidence therefore suggests that the secondary rise in portal pressure after acetylcholine is dependent on the integrity of the sympathetic system. When this is thrown out of action by ergot the rise does not take place. The secondary rise does not occur when the sympathetic tone has been increased as a result of a failing circulation.

It is also to be noted that in the animal with paralysis of the sympathetic system, the rate of recovery of the arterial pressure from the acetylcholine depression is slower than in the animal with normal circulatory reactions, such as that in Figs. 4 and 5. The recovery rate is also

slow in the presence of a failing circulation. The association of the period of rapid recovery (Fig. 7), with a diminution of flow through the splanchnic arterioles, is evidence of splanchnic vaso-constriction at this stage. All these points are in favour of the hypothesis that the sympathetic plays a part in the recovery of the blood-pressure after acetylcholine. Depending on the degree of sympathetic stimulation elicited, so does the secondary rise in portal pressure vary in extent.

Thus acetylcholine resembles adrenaline in calling out the opposing autonomic mechanism at the height of its action. Reflex vaso-dilatation and cardiac slowing are elicited at the point of maximum adrenaline effect. Acetylcholine at its point of maximum activity calls forth cardiac acceleration and splanchnic vaso-constriction.

Discussion.

The results obtained in this investigation add some confirmatory evidence to the view of Griffith and Emery [1930] that the vagus nerve does not carry vaso-motor fibres to the liver. These observers stimulated the vagus nerve, avoiding cardiac inhibition, and found no change in liver volume. The experiment described in Fig. 19 would indicate too, that as far as the portal vein is concerned there are no sympathetic dilator fibres. The vaso-dilator reflexes in the liver obtained by Griffith and Emery, were shown to be mediated through the splanchnics, and it is probable that any dilator reaction which occurs in the portal venules must be due to inhibition of vaso-constrictor tone.

Effects similar to those resulting from the intravenous injection of acetylcholine were obtained by Carnot, Gayet and Merklen (1930), by stimulation of the vagus. They found an initial fall in portal pressure, which they ascribed to diminished inflow into the portal system, and a rise of portal pressure, which occurred as the arterial pressure recovered.

So far as the cat is concerned, there are anatomical reasons brought forward by Popper [1931], which indicate that the "Lebersperre" mechanism described in the dog by Mautner and Pick, and by Bauer, Dale, Poulsson and Richards [1932] is absent. In the dog the muscular coat of the hepatic vein is much better developed than that in the cat, or the human subject. Dale and his colleagues did not find any clear evidence of vagus control of the liver circulation nor did they record any significant results following the injection of acetylcholine. Grab, Janssen and Rein [1929–30], showed an increased rate of flow through the liver with atropine. Inflow and outflow were simultaneously increased, and

thus there is no reason to assume any immediate vagus effect upon the liver circulation. Such effects as are observed are entirely secondary to alterations in the general circulation.

In the experiments detailed in this and in the preceding paper [1932], outflow from the liver has not been measured directly. All the vaso-motor reactions which we have obtained have been satisfactorily accounted for by the demonstrable changes in the circulation through the splanchnic vessels and the portal vein. In none of the experiments has there been any evidence of a controlling mechanism at the outlet of the liver lobule. This is in complete agreement with the observations made by Dale and his collaborators on the liver of the cat.

Summary.

In the cat:

1. Large doses of acetylcholine bring about a rise in portal pressure by constricting the portal vein.

2. (*a*) With smaller doses of acetylcholine there is no direct constricting action on the portal vein. Two phases are usually seen in the portal pressure curve, viz. an initial fall, and a secondary rise of pressure.

(*b*) The initial fall in portal pressure is determined by diminished inflow into the portal system, due to the predominance of vaso-dilatation in the limbs, and to diminished cardiac output.

(*c*) The diminished flow through the splanchnic arterioles, and the consequent fall in portal pressure, can be reversed by the Bayliss mercury compensator.

(*d*) During the stage of cardiac slowing, the general venous pressure may rise. This rise is exaggerated in animals which have been rendered plethoric; in such animals the portal pressure may rise in parallel with the general venous pressure, and the initial portal pressure fall is not seen.

(*e*) The secondary rise in portal pressure is due to constriction of the portal venules within the liver by the action of the sympathetic. It is absent when the sympathetic has been paralysed by ergot, and in the presence of a failing circulation.

3. There is no evidence of any parasympathetic dilator action on the portal or hepatic venules. The experiments with ergot also yielded no evidence of sympathetic dilator fibres to the portal vein.

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THE NERVOUS CONTROL OF THE CAUDAL REGION OF THE LARGE BOWEL IN THE CAT.

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To find the influence of a nervous outflow on an organ in the body one may either cut the nerve, with subsequent stimulation of the cut ends, or one may simply divide the nerve. In the former case the response of the organ is to nerve impulses set up by an abnormal stimulus which may elicit impulses in quantity and in quality different from the normal, while in the latter case, after the disturbances due to trauma have subsided, the response exhibited by the viscus is due to the absence of normal impulses.

The latter method has found little favour in the past. Barrington [1915], however, used just such a method, often in acute preparations, to elucidate the normal nervous control of the urinary bladder. The close developmental and physiological connection between the large bowel and the urinary bladder suggests that a similar method may be successful in investigating the normal nervous control of the large bowel.

Nomenclature.

Because of present uncertainty about the constitution and function of the nervous outflows to the large bowel the purely morphological terms "lumbar" and "sacral" are used in preference to "sympathetic" and "parasympathetic" respectively. The names applied to the subdivisions of the outflows are those recommended by Langley and Anderson [1896]. The lumbar outflow arises from the second, third, fourth and sometimes fifth lumbar nerves. The fibres pass without interruption through the sympathetic chains and run as four or five strands on either side to the inferior mesenteric ganglia. These strands are the spinal rami of the inferior mesenteric ganglia. The inferior mesenteric ganglia, usually four in number, form a ring round the inferior mesenteric artery about 1½ cm. from the origin of the artery from the aorta. The majority of the fibres of the lumbar outflow form synapses in these ganglia. From the ganglia arise the lumbar colonic nerves which accompany the inferior mesenteric artery to the colon. There also arise from the inferior mesenteric ganglia the hypogastric nerves which run caudad to join the pelvic plexus.

The sacral outflow consists of the pelvic nerves which usually arise from the second and third sacral nerves. The pelvic nerves divide after a short course into a cranial and caudal branch on each of which there is a conspicuous ganglion. From the caudal ganglion arise four or more branches which run dorsad to the colon as the sacral colonic nerves. The fibres of the sacral outflow have synapses close to the bowel itself.

Fig. 1, drawn from the description by Langley and Anderson [1896], illustrates diagrammatically the outflows to the large bowel.

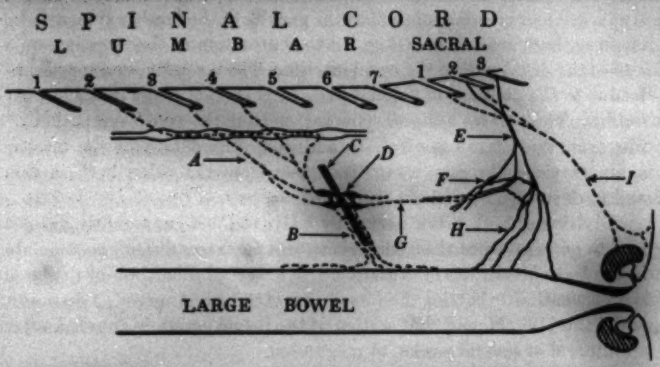

Fig. 1. Diagram of the outflows to the large bowel in the cat. *A*, spinal rami to the inferior mesenteric ganglia; *B*, lumbar colonic nerves; *C*, inferior mesenteric artery; *D*, inferior mesenteric ganglia; *E*, pelvic nerve; *F*, pelvic plexus; *G*, hypogastric nerve; *H*, sacral colonic nerves; *I*, pudendal nerve.

Previous work.

Bayliss and Starling [1900] and Elliott and Barclay-Smith [1904] found that the colon was always inactive after opening the peritoneum. Pithing the lumbo-sacral cord or dividing the nervous outflows to the large bowel led to activity of the gut. Lehmann [1913] noted increased colonic movement in dogs after division of the spinal rami to the inferior mesenteric ganglia. Markowitz and Campbell [1927] showed that spinal anæsthesia in dogs removes inhibition of the small intestine produced by intense peritoneal irritation, and in one of their five cases they thought that the movements of the colon were also augmented. Learmonth and Markowitz [1930] found that division of the lumbar colonic nerves in dogs increased colonic activity. This increase in activity was apparently greater when the pelvic nerves were intact. In these experiments, unfortunately, the peritoneum had been opened before section of the lumbar colonic nerves. Rankin and

Learmonth [1930] recommend division of the inferior mesenteric (lumbar colonic) nerves and of the presacral (hypogastric) nerves in man for the relief of megacolon.

Kuré [1931] and his fellow workers, however, cut the rami communicantes to the lumbar sympathetic chain on both sides in four dogs. The caudal part of the small intestine and the large intestine relaxed. Application of nicotine solution to the ganglia of the lumbar sympathetic chain on both sides in four dogs led to contraction of the large intestine and of the distal part of the small intestine. These results are supposed to be due to the presence of "spinal parasympathetic" fibres in the lumbar outflow. These fibres have no synapses, as have the true "sympathetic" fibres, in the lateral ganglia. In addition, stimulation of the lumbar sympathetic chain gave motor responses from the colon both in cats and in dogs with or without the use of nicotine.

Division of both pelvic nerves by Elliott [1906] apparently did not lead to gross atony of the colon in cats because faeces did not accumulate in the large bowel. Barrington [1915] observed dilatation of the large bowel in cats after cutting the dorsal roots of the sacral nerves. Adamson and Aird [1932] found that section of the pelvic nerves in cats led, after an interval of several weeks, to megacolon.

Methods.

The cats were decerebrated under ether anæsthesia and no observations were carried out for at least 1 hour after decerebration. In a few cases decapitation or anæsthesia with one of the barbituric derivatives was substituted for decerebration. Faecal matter was removed from the large bowel after decerebration by means of a soap and water enema. The animals received a meal of porridge and milk on the morning of the day of the experiment some 2 hours before anæsthetization.

The behaviour of the large bowel was recorded by a thin rubber balloon of large diameter inserted through the anus. The balloon holder, 22 cm. long, was a thin copper tube with numerous perforations in the terminal 4 cm. where the balloon was attached. The copper tube was marked off in cm. so that the position of the balloon within the gut could be gauged. Usually the caudal end of the balloon lay from 4 to 6 cm. craniad to the anal canal. The balloon holder was firmly grasped by a clamp attached to the operating table.

Volume recording at a constant pressure of 21 cm. water was used. The tonicity of the gut was estimated by recording the volume of fluid

accepted by the balloon in the gut at constant pressure. The rhythmical behaviour of the gut appeared on the record after release of the pressure. Several consistent records were obtained before interfering with the nervous outflows to the gut.

The lumbar outflow was approached extraperitoneally through a left flank incision. The hypogastric nerves were cut without opening the peritoneum, but the peritoneum had to be opened in order to divide the spinal rami to the inferior mesenteric ganglia and also to cut the lumbar colonic nerves. The pelvic nerves were exposed and cut extraperitoneally by incisions along the inguinal canals.

The lumbo-sacral cord was isolated by tying the spinal cord in the lower thoracic region. Full ether anæsthesia was used and ample time allowed for disappearance of the ether before continuing the observations. Such cord section is craniad to the roots of origin of the lumbar and sacral outflows in cats.

Spinal anæsthesia was induced by intrathecal injection of 1 to 2 c.c. of a 1 p.c. "Novocain" solution craniad or caudad to the last lumbar spinous process. As tested by the knee jerk, anæsthetization of the cord lasted for from 30 to 60 minutes.

Results.

(1) *The effect of division of the lumbar outflow.* When the lumbar outflow is intact the large bowel is inactive. Section of the entire lumbar outflow leads to increase in tone and in rhythmical activity (Fig. 3, C, 1, 2). Section of the spinal rami to the inferior mesenteric ganglia alone causes a slight increase in tone and the appearance of rhythmical contraction. Subsequent section of the lumbar colonic nerves and of the hypogastric nerves leads to marked increase both in tone and in rhythmicity (Fig. 2, B). Division of the hypogastric nerves alone has little effect, but section of the lumbar colonic nerves, even when the hypogastric nerves are intact, leads to this marked increase in gut activity (Fig. 2, A, C, 1, 2, 3). Such results occur after isolation of the lumbo-sacral cord and the integrity of the pelvic nerves seems to make little difference to the result.

(2) *The effect of division of the sacral outflow.* In five cats the sacral nerve roots or the pelvic nerves themselves were cut and the animals allowed to recover. These animals experienced difficulty in emptying the large bowel and post-mortem the colon was dilated.

In acute preparations, when the lumbar outflow is intact, division of the pelvic nerves may lead to further relaxation of the large bowel

(Fig. 3, A, 2, 3). Such relaxation is rarely marked but, when the lumbar outflow has previously been cut, and the gut in consequence in a state

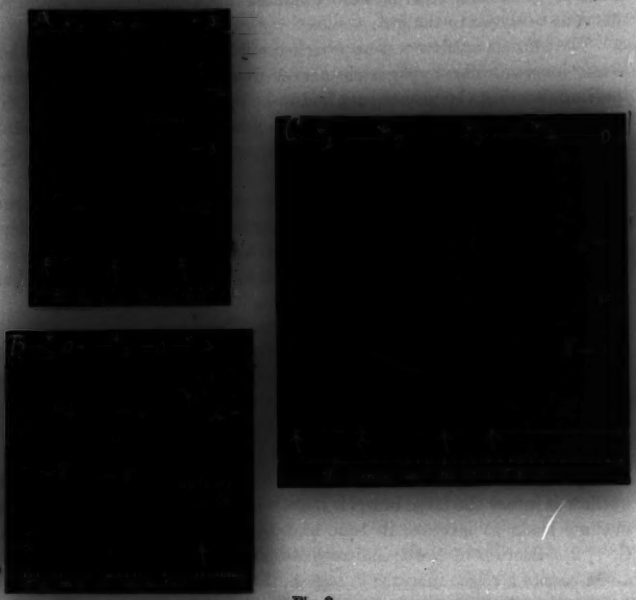

Fig. 2.

Series A. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H_2O. 2. Lumbar colonic nerves cut; pressure released as before. 3. Hypogastric nerves cut; pressure released as before.

Series B. Cat: decerebrate; pudendal nerves cut; lumbo-sacral cord isolated; pelvic nerves cut. 1. Pressure released at 21 cm. H_2O. 2. Spinal rami to inferior mesenteric ganglia cut; pressure released as before. 3. Lumbar colonic and hypogastric nerves cut; pressure released as before.

Series C. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H_2O. 2. Hypogastric nerves cut; pressure released as before. 3. Lumbar colonic nerves cut; pressure released as before. 4. Pelvic nerves cut; pressure released as before.

of high tone and activity, section of the pelvic nerves then produces an unequivocal fall in tone and possibly some decrease in the rate of rhythmical contraction (Fig. 2, C, 3, 4). After the division of the pelvic nerves the decrease in resistance to passage of the recording balloon craniad

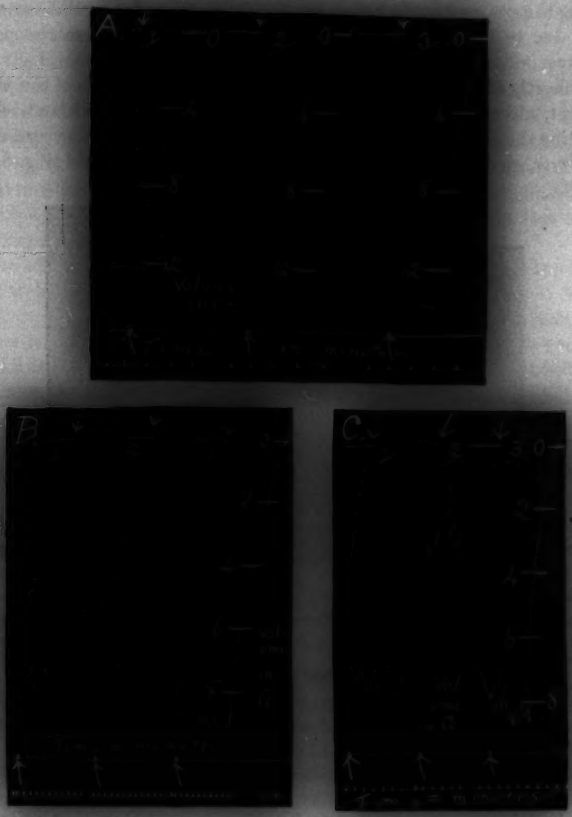

Fig. 3.

Series A. Cat: decerebrate. 1. Pressure released at 21 cm. H_2O. 2. Pudendal nerves cut; pressure released as before. 3. Pelvic nerves cut; pressure released as before.

Series B. Cat: decerebrate; lumbar outflow cut; lumbo-sacral cord isolated. 1. Pressure released at 21 cm. H_2O. 2. Pelvic nerves exposed; pressure released as before. 3. Pelvic nerves cut 3 hours after cord transection; pressure released as before.

Series C. Cat: decerebrate; pudendal nerves cut. 1. Pressure released at 21 cm. H_2O. 2. Lumbar outflow cut; pressure released as before. 3. Cord tied in lower thoracic region; pressure released as before.

along the gut may be very obvious. The fall in tone of the gut is certainly not due to the operative interference necessary to expose the pelvic nerves.

Shortly after isolation of the lumbo-sacral cord, division of the pelvic nerves, although the lumbar outflow be cut, is usually without effect. Cutting the pelvic nerves several hours after transection of the cord may, however, give some indication of relaxation of the large bowel (Fig. 3, B).

Fig. 4.

Series A. Cat: Na amytal anæsthesia-55 mg./kg.-pudendal nerves cut: pelvic nerves cut. 1. Pressure released at 21 cm. H_2O. 2. Cord tied in lower thoracic region; pressure released as before.

Series B. Cat: Na amytal anæsthesia-56 mg./kg. 1. Pressure released at 21 cm. H_2O. 2. 1 c.c. 1 p.c. "Novocain" injected intrathecally; pressure released as before 10 min. after injection. 3. Pressure released as before 60 min. after injection.

(3) *The effect of isolation of the lumbo-sacral cord.* When the lumbar outflow is intact and the pelvic nerves divided, isolation of the lumbo-sacral cord causes no significant alteration in the large bowel; there is certainly no contraction (Fig. 4, A). When, however, the lumbar outflow is cut and the sacral outflow intact, transection of the spinal cord leads to obvious relaxation of the large bowel (Fig. 3, C, 2, 3). Even when both outflows are intact, isolation of the lumbo-sacral cord may cause slight relaxation of the large bowel.

(4) *The effect of spinal anæsthesia.* When both outflows to the large bowel are intact, spinal anæsthesia invariably leads to increased activity

of the gut. The result is similar after division of the sacral outflow and after isolation of the lumbo-sacral cord.

If the lumbar outflow be cut and the sacral outflow left intact, spinal anæsthesia leads to relaxation of the gut, very apparent before section of the cord but slight after isolation of the lumbo-sacral cord. The possibility that spinal anæsthesia indirectly affects the volume of fluid accepted by the gut by causing flaccidity of the abdominal and pelvo-caudal muscles throws some doubt on such observations, although, after division of both outflows to the large bowel, spinal anæsthesia does not have any obvious effect on the record from the gut.

The effect of spinal anæsthesia on the large bowel of an anæsthetized cat with both outflows intact is shown in Fig. 4, B.

When both outflows to the gut are intact, or when only the lumbar outflow is intact, division of the pudendal nerves, in practically every case, leads to slight but definite increase in the activity of the large bowel (Fig. 3, A, 1, 2).

Discussion and conclusions.

The lumbar outflow exerts a constant inhibitory influence on the large bowel, and this inhibition is apparently more powerful than the motor tone passing by the sacral outflow. If the peritoneal cavity be opened beforehand, such powerful inhibition is understandable, and division of any part of the lumbar outflow can give no true indication of the normal impulses passing along the lumbar outflow. In the present series, however, the integrity of the peritoneal cavity was jealously guarded before division of the lumbar outflow. It may be that the marked inhibition is due to the recent operative interference in acute preparations. On the other hand, the motor influence through the sacral outflow may be intermittent, as the work of Hertz and Newton [1913] on the gastro-colic reflex suggests. Such intermittent activity, however powerful, simple nerve section cannot be expected to detect.

A constant motor influence of the sacral outflow, however, may be detected by previous division of the lumbar outflow, but this does not seem to be powerful in acute experiments, and, even in survival experiments, division of the sacral outflow apparently does not lead to rapid or gross atony of the large bowel. It is possible that the dilatation of the large bowel observed by Barrington [1915] and by Adamson and Aird [1932], after interference with the sacral outflow, is due to distension of the gut as the result of the absence of the normal expulsive responses which depend so largely on the integrity of the pelvic nerves [Garry, 1932].

The central inhibitory tone for the large bowel seems to be confined to the lumbo-sacral cord. In this the colon differs from the urinary bladder where part of the inhibition in the lumbar outflow arises craniad to the lumbar cord [Barrington, 1915]. It is difficult to decide if the inhibitory influence of the inferior mesenteric ganglia is a normal phenomenon or not. Barrington [1915] also found that the inferior mesenteric ganglia act as an extraspinal centre of inhibition for the bladder. Division of the spinal rami to the inferior mesenteric ganglia alone certainly never causes such marked increase in colonic activity as division of the lumbar colonic nerves. This may be due to failure to cut all the spinal rami to the inferior mesenteric ganglia, but it is possible that the inhibitory influence exerted by such an extraspinal centre is the result of the local interference at the time of section of the spinal rami. The marked increase in gut activity following spinal anæsthesia, in effect division of the spinal rami without local interference, suggests that the inhibitory powers of the inferior mesenteric ganglia are acquired in response to an abnormal situation. Distal to the inferior mesenteric ganglia the inhibitory impulses follow the lumbar colonic nerves almost exclusively. The persistence of the inhibition after division of the hypogastric nerves is not due to switching of impulses, normally passing down the hypogastric nerves, to the lumbar colonic nerves, because initial division of the lumbar colonic nerves leads to an increase in bowel activity little, if at all, augmented by subsequent section of the hypogastric nerves. Stimulation of the peripheral ends of the cut hypogastric nerves causes obvious inhibition of the colon which shows the danger of drawing conclusions from the results of nerve stimulation alone.

The motor influence through the sacral outflow arises mainly craniad to the lumbo-sacral cord. There is some slight evidence, even in acute preparations, for a secondary motor centre in the isolated lumbo-sacral cord. Such conclusions are similar to those of Barrington [1915] for the urinary bladder.

The slight increase in activity of the large bowel after division of the pudendal nerves may be due to removal of afferent impulses in the pudendal nerves set up by irritation of the anal canal. Such impulses, like other afferent impulses, may tend to cause inhibition of the gut.

SUMMARY.

The behaviour of the caudal end of the large bowel in cats is recorded by means of a balloon introduced through the anus.

In acute decerebrate cats the gut is invariably inactive. This inactivity is due to inhibitory impulses, arising locally in the lumbo-sacral cord, passing out to the large bowel by way of the spinal rami to the inferior mesenteric ganglia and then by the lumbar colonic nerves. The hypogastric nerves do not normally transmit such impulses to the gut. After section of the spinal rami the inferior mesenteric ganglia still exert an inhibitory influence on the large bowel. The motor influence exerted by the sacral outflow is not strong and arises mainly craniad to the lumbo-sacral cord. There is some evidence for a subsidiary motor centre in the isolated lumbo-sacral cord.

Division of the pudendal nerves causes a slight increase in the activity of the large bowel.

I am deeply indebted to Prof. E. P. Cathcart for constant interest and encouragement in this work.

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THE INFLUENCE OF OXYGEN PRESSURE ON THE METABOLISM OF THE ISOLATED COLD-BLOODED HEART.

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The authors found that the respiratory quotients of isolated hearts of frogs and of tortoises were lower when the hearts were suspended in pure oxygen than when suspended in air. Furthermore the R.Q. in oxygen in most cases was less than 0·8, a fact which suggested the possibility of the hearts utilizing fat. The experiments described below were made in order to analyse the metabolic exchange of hearts working in air and in oxygen, in the hope of explaining these phenomena.

Methods.

Rana esc. (Hung.) and *Testudo graeca* were used. The oxygen consumption and carbon dioxide production were measured by the method already described by the authors [1931]. The Barcroft apparatuses used had large bulbs with a capacity of about 400 c.c. and two or three frogs' hearts or one tortoise heart were used in each experiment. The frogs' hearts were perfused by the method previously described [1931] and the tortoise hearts were perfused by a similar method, with two cannulae (venous and aortic) which were arranged to provide a circulation. In some experiments the tortoise auricle alone was used, and was prepared by the method described by Clark and White [1930]. The Ringer's fluid used was buffered with phosphate, and was of the composition previously described [1931].

The general method adopted was to allow 2 hours for equilibrium to be established after the hearts had been set up in the Barcroft apparatus, and to take readings for the subsequent 4 hours. In order to estimate the total oxygen usage, the oxygen consumption during the first 2 hours was

[1] Beit Memorial Research Fellow.

[2] In receipt of a part-time grant from the Medical Research Council.

calculated by extrapolation from the later figures. It was found that irregular readings were obtained for both oxygen consumption and CO_2 production during the first hour, but that between the first and second hours both these figures attained steady values; these were maintained until the end of the experiment provided that the hearts remained in good condition. Experience showed that reliable measurements of the R.Q. could be obtained if the hearts maintained a fairly uniform activity for 6 hours, but that with the large bulbs used in these experiments readings over shorter periods gave unreliable results. The values obtained for the R.Q. also were found to be unreliable when there was any progressive impairment of the hearts' activity, such as was produced by oxygen lack or by poisoning with iodo-acetic acid.

The chemical methods used have been described in previous papers by the authors: estimation of total carbohydrate [1931]; estimation of lactic acid [1932 *b*]; a new method was used for the estimation of fat, namely that described by Stewart, Gaddie and Dunlop [1931]. This is a titrometric method that estimates the number of carboxyl groups in precipitable fatty acids.

The effect of oxygen pressure on the respiratory exchange.

The first object of the experiments was to determine the effect on the R.Q. of substituting pure oxygen for air. In a series of experiments all conditions were kept exactly constant, except that on alternate days the Barcroft bulbs were filled with pure oxygen instead of air. Any differences observed cannot therefore be due to any constant experimental error, nor to any change in the condition of the frogs.

Table I shows the fundamental result dealt with in this paper. In the four sets of experiments shown in the table, the R.Q. was in every case lower in oxygen than in air, and the difference was considerable (from 0·17 to 0·12).

The figures obtained in air may be compared with the following figures previously obtained by the authors, with frogs' hearts perfused for 6 hours and suspended in air. (The values for oxygen consumption have been converted to c.c. per g. per hour.) Perfused Ringer's fluid: (1931) 21 hearts, 1·26 c.c. O_2 (R.Q. 0·86); (1932 *a*) 11 hearts, 1·39 c.c. O_2 (R.Q. 0·89). Perfused Ringer's fluid with 25 p.c. serum: (1931) 8 hearts, 1·9 c.c. O_2 (R.Q. 0·84). Perfused with heparinized frog's blood: (1931) 7 hearts, 1·51 c.c. (R.Q. 0·90).

The present results show a lower oxygen consumption in Ringer's fluid, and a slightly higher R.Q. than do our previous figures. The only other difference worth noting is that in our previous work we found a slightly lower R.Q. with Ringer serum than with Ringer's fluid, whereas the present results show the former figure slightly greater than the latter. The two sets of results indicate that there is no significant difference in the R.Q. in Ringer's fluid with and without the addition of plasma or serum.

TABLE I. Respiratory exchange in air and in oxygen.

(Duration of experiments 6 hours.)

System	Air				Oxygen			
	No. of hearts	R.Q.	Oxygen used (c.c. per g. per hr.)	CO_2 produced (c.c. per g. per hr.)	No. of hearts	R.Q.	Oxygen used (c.c. per g. per hr.)	CO_2 produced (c.c. per g. per hr.)
Frogs' hearts.								
1. Ringer's fluid	34	0·903	0·80	0·72	39	0·730	0·90	0·66
2. Ringer's fluid + 10 p.c. frog's plasma (heparinized)	12	0·913	1·18	1·08	24	0·760	1·35	1·03
3. Ringer's fluid + 50 p.c. frog's blood (heparinized)	8	0·902	1·92	1·73	12	0·793	2·28	1·81
Tortoises' hearts.								
Ringer's fluid + 10 p.c. tortoises' serum	5	1·035	0·46	0·50	4	0·915	0·69	0·63

The figures for frogs' hearts in Table I show the effect of substitution of pure oxygen for air in hearts perfused with Ringer's fluid, with Ringer plasma, and with Ringer blood. This change caused an increase of oxygen consumption in all cases, whilst the CO_2 production decreased about 5 p.c. in two cases and increased about 5 p.c. in the third case. In the case of the tortoise heart both the oxygen consumption and the CO_2 production were increased considerably by substitution of pure oxygen for air.

These results indicate that oxygen causes the hearts to metabolise some substance with a low R.Q. The chief probable source of error affecting this conclusion is a variable production of lactic acid, for the acid might liberate preformed CO_2 from the system, and if more acid were produced in air than in oxygen this might account for some or all of the differences observed in the R.Q.

LACTIC ACID PRODUCTION.

The perfusion fluid when introduced contained no carbonate but Clark and White [1928*b*] found that during perfusion an exchange of ions took place between the heart and the perfusion fluid; either phosphate or chloride ions passed into the heart and were replaced by carbonate ions. The present authors found [1931, Table XIII] that frogs' hearts after perfusion with phosphate Ringer's fluid for 6 hours lost about

0·15 c.c. CO_2 per g., and that the perfusion fluid took up about 0·25 c.c. CO_2 per g. of heart. In our present experiments readings were commenced after 2 hours' perfusion, and at this time sufficient CO_2 would be present in the fluid for a measurable amount to be liberated by any lactic acid produced.

TABLE II. Lactic acid produced in 6 hours by perfused hearts.

	Air				Oxygen			
System	No. of hearts	Lactic acid produced (mg. per g.)	CO_2 equivalent of lactic acid produced (c.c. per g.)	Total CO_2 produced (c.c. per g.)	No. of hearts	Lactic acid produced (mg. per g.)	CO_2 equivalent of lactic acid produced (c.c. per g.)	Total CO_2 produced (c.c. per g.)
			Frogs' hearts					
1. Ringer's fluid	20	0·564	0·14	4·32	22	0·296	0·074	3·96
2. Ringer's fluid + 10 p.c. frogs' plasma	8	0·550	0·14	6·48	8	0·530	0·130	6·18
			Tortoises' hearts					
Ringer's fluid + 10 p.c. tortoises' serum	10	2·180	0·54	3·00	8	0·156	0·039	3·78

Table II shows the amount of lactic acid excreted by hearts under various conditions. The authors have shown [1932 *a*] that the lactic acid content inside the heart does not rise during perfusion. The maximum amounts of CO_2 that could be liberated by the lactic acid excreted are shown in Table II. These quantities are calculated from the formula

$$1 \text{ mg. lactic acid} = \frac{22{\cdot}4}{90} = 0{\cdot}25 \text{ c.c. } CO_2.$$

With regard to the figures obtained it may be noted that the value of 0·564 mg. lactic acid produced per g. heart in 6 hours obtained with hearts perfused with Ringer's fluid in air, is higher than the previous figure of 0·6 mg. per g. in 20 hours obtained by us (1932 *b*) with hearts perfused in the same manner. The significance of the figures for lactic acid production in hearts perfused with plasma is somewhat uncertain, for some production occurred in control fluids kept standing at room temperature for 6 hours.

Table II shows that in the case of frogs' hearts the lactic acid production in either air or oxygen did not exceed 0·56 mg. per g. and that the amount of CO_2 that this quantity could liberate was not more than about 3 p.c. of the total CO_2 production of the heart. Moreover the lactic acid production in air and oxygen was equal in the case of plasma perfusion, and in the case of Ringer perfusion the difference was only 0·27 mg. per g. In the case of the frogs' hearts therefore lactic acid

production could not produce any measurable error in the R.Q. estimations.

In the case of the tortoises' hearts there is a large difference in the lactic acid production in air and in oxygen. Subtraction of the CO_2 that might be liberated by the lactic acid would reduce the R.Q. observed in air from 1·035 to 0·892, a figure which is nearly the same as the R.Q. obtained in oxygen which is 0·903 when similarly corrected. Lactic acid production therefore provides an adequate explanation for the fact that an R.Q. above unity was obtained with the tortoise heart in air, and might account for the difference observed in the R.Q.'s obtained in air and in oxygen. The experiments on tortoises' hearts do not therefore provide conclusive evidence of a difference in the R.Q. in air and in oxygen, but in the case of frogs' hearts, the difference observed in these two conditions is not affected significantly by the lactic acid production.

Carbohydrate metabolism in air and in oxygen.

Estimations of the carbohydrate consumption of isolated hearts must be based on a comparison of the amount of total reducing substances present in hearts after perfusion with the amount present in unperfused controls. Unfortunately it is very difficult to obtain reliable control values, for the authors have found that the values obtained in the controls vary greatly not only as regards individual frogs, but equally as regards the averages of different batches, and also in the same batches of frogs after different periods of captivity. For example after a few weeks' captivity the amount of reducing substance in control hearts rises rapidly. Hence it not only is necessary to use large numbers of frogs as controls, but also it is necessary to make control estimations at the same time as the experiments are carried out. We have taken as full precautions as possible but feel that calculations based on comparisons with controls are somewhat uncertain.

The estimations of the content of total reducing substances in controls, shown in Table III were obtained from the following numbers: batch I, 19 hearts; batch II, 6 hearts; batch IV, 12 hearts. The standard deviation of the mean (σ_m) varied between 0·6 and 0·8. In the case of the averages for the perfused hearts, the σ_m lay between 0·5 and 1.

The figures for lactic acid excretion are taken from Table II. The figures for sugar excretion agree with the values we have obtained previously [1932 *a*, Table III]. As regards the calculations of gain and loss of reducing substances, the estimations of the standard variation of the averages indicates that differences of less than 2 mg. per g. are of doubtful significance.

The results shown in Table III provide fairly certain evidence that the hearts use more carbohydrate when working in air than when working

in oxygen. In the first place the R.Q. is higher in air than in oxygen, and in the second place all four pairs of averages given in Table III show a lower value for the total reducing substances in the heart after perfusion in air than after perfusion in oxygen. In all four cases the experiments compared were conducted on alternate days, and therefore the differences observed are believed to be significant.

TABLE III. Carbohydrate metabolism of frogs' hearts.

Batch no. of frogs	No. of hearts	Duration of exp. in hr.	Total O_2 used (c.c. per g. heart)	R.Q.	Total reducing substance in hearts (mg. per g.)		Amounts excreted into (+) or removed from (−) perfusion fluid (mg. per g. heart)		Loss or gain of carbohydrate in the heart-fluid system (mg. per g. heart)
					(*a*) Controls	(*b*) Perfused	(*a*) Lactic acid	(*b*) Sugar	
Perfused with Ringer's fluid in air.									
I *a*	9	6·0	4·50	0·93	15·22	12·48			−1·10
II *a*	9	6·0	5·90	0·87	8·90	5·71	+0·56	+1·08	−1·55
III *a*	16	6·3	4·46	0·90	—	7·75			—
Perfused with Ringer's fluid in oxygen.									
I *b*	12	6·0	4·33	0·74	15·22	15·49			+2·09
II *b*	15	6·0	6·15	0·75	8·90	7·31	+0·30	+1·34	+0·05
III *b*	12	7·3	6·66	0·70	—	7·97			—
Perfused with Ringer plasma in air.									
IV *a*	12	6·0	7·10	0·91	14·99	7·78	+0·55	−0·04	−6·60
Perfused with Ringer plasma in oxygen.									
IV *b*	12	6·0	7·16	0·74	14·99	10·41	+0·52	−0·10	−4·16
Perfused with Ringer's fluid in 95 p.c. N_2 and 5 p.c. O_2.									
II *c*	12	6·0	2·94	—	8·90	5·66	+1·0	+1·84	−0·40
Perfused in oxygen with Ringer's fluid +0·001 p.c. iodo-acetic acid.									
II *d*	27	6·0	2·75	—	15·22	12·86	0	+1·78	−0·58

The estimations of total reducing substances indicate that hearts perfused with Ringer's fluid used very little carbohydrate when perfused in air, and the figures for the hearts perfused in oxygen actually show a gain in reducing substances. The significance of this apparent gain is uncertain but the problem has been discussed previously by the authors [1932 *a*].

A few experiments were made with reduced oxygen pressure (5 p.c. O_2 in N_2). These were inconclusive because heart block developed after a few hours. The writers have shown [1931] that a ventricle driven by electrical stimuli can maintain its activity unimpaired for hours under anaerobic conditions. This, however, is not true as regards the conductive mechanism of the heart which is very easily paralysed by deficiency of oxygen. Similarly iodo-acetic acid in low concentrations produced heart block after 2–3 hours. Clark, Eggleton and Eggleton [1932] found that the frog's ventricle, driven by electrical stimulation, maintained a

normal activity in air for 2 hours in the presence of 0·008 p.c. iodo-acetic acid. This acid appears therefore to poison the conductive mechanism of the frog's heart long before it affects the contractility of the ventricle. The experiments with iodo-acetic acid show very little change in the reducing substances of the heart, whilst the experiments with reduced oxygen pressure show a considerable excretion of lactic acid. Unfortunately in both cases the occurrence of heart block made it impossible to get reliable figures for the R.Q.

Carbohydrate metabolism of tortoise heart.

Experiments were made with tortoise hearts in the hope that the use of a larger mass of material would lead to increased accuracy of analysis. On the whole the tortoise heart has been found less suitable than the frog's heart for the purposes of these experiments. The heart provided with circulation does not obtain an adequate oxygen supply when working in air, but appears to do so when suspended in oxygen. The isolated auricle filled with Ringer serum mixture and suspended in oxygen probably does not obtain an adequate oxygen supply. Unfortunately the tortoises' hearts vary even more widely than do frogs' hearts as regards their content of reducing substances.

The content of reducing substances was estimated in the unperfused ventricles of 18 tortoises from a single batch and the average value obtained was 17·7 mg. per g., but the individual values varied from 8·2 to 30·8. The standard deviation was 6·7 and the σ_m was 1·6. There was however a correlation between the content of reducing substances in the auricle and in the ventricle. These two values were estimated in 9 hearts and the average figures were auricle 15 and ventricle 16·7 mg. reducing substance per g., whilst the average divergence between auricle and ventricle was only 2·7 mg. The control values for total reducing substances shown in Table IV were obtained as follows. In the 1930 experiments the auricles were perfused and the ventricles analysed as controls. The figure for 1931 is the average of 21 estimations. In the case of the 1932 experiments the number of tortoises available was not sufficient for control estimations and the value of 23·3 is the average of the 10 highest values in the previous batch, and merely indicates the highest probable figure.

The experiments made in 1932 on tortoises' hearts (Table IV) confirm the experiments on frogs' hearts in that the hearts suspended in air show a lower content of reducing substances than do those suspended in oxygen. About half of this difference can be accounted for by the lactic acid produced by hearts working in air. The figures indicate that the tortoise heart working in oxygen uses little carbohydrate but uses more when working in air. The auricle suspended in oxygen may metabolize as much as two-thirds of its carbohydrate but we believe that the

tortoise's auricle even when suspended in oxygen suffers from oxygen lack. The results do not indicate any important difference between the metabolism of the tortoise's heart and that of the frog's heart.

TABLE IV. Carbohydrate metabolism of tortoises' hearts.

Date	No. of hearts	Duration of exp. in hr.	Total O_2 used (c.c. per g. heart)	R.Q.	Total reducing substances in hearts (mg. per g.) (*a*) Control	(*b*) Perfused	Amounts excreted into (+) or removed from (−) perfusion fluid (mg. per g. heart) (*a*) Lactic acid	(*b*) Sugar	Loss or gain of carbohydrate in heart fluid system (mg. per g. heart)
Whole heart perfused Ringer serum in air.									
1932	5	6	2·74	1·035	(23·3)	15·1	+2·46	+0·03	5·7
Whole heart perfused Ringer serum in oxygen.									
1932	4	6	4·16	0·915	(23·3)	20·4	+0·17	+0·01	2·7
Whole heart perfused Ringer's fluid in oxygen.									
1931	7	24	11·3	—	16·3	12·7	—	—	3·4
Auricle filled with Ringer serum in oxygen.									
1930	7	24	10·5	—	18·0	17·3	—	−2·0	>11

NITROGENOUS METABOLISM.

Table V shows the amount of non-protein nitrogen excreted into perfusion fluids by the hearts. The values are very similar to those obtained previously by the authors, who found that the nitrogen excretion by frogs' hearts perfused with Ringer's fluid in air for 6 hours was 0·52 mg. per g. [1931], and 0·42 mg. per g. [1932 *a*]. The nitrogen excretion in plasma appears to be lower than that in Ringer's fluid, but the values with plasma are obtained by difference between the nitrogen content of control and perfusion fluid, and are therefore less accurate than those with Ringer's fluid.

The tortoise's heart, on account of its greater bulk, provides a more favourable opportunity for measuring nitrogen excretion, and it will be seen that this is only half as great per unit weight with the tortoises' hearts as with frogs' hearts. A control experiment showed that the tortoise heart (unperfused) contained 0·12 mg. per g. of urea N. The quantity of nitrogen found in the perfusion fluid was considerably greater than this, and must therefore have been formed during perfusion and cannot have been simply washed out of the heart.

We have previously estimated [1932 *a*] that about 0·07 mg. per g. of nitrogen is excreted for every c.c. of oxygen consumed. If it be

assumed that the excretion of 1 mg. N_2 involves an oxygen use of 6 c.c., then the nitrogen excretions shown in Table V account for proportions of the total metabolism which vary from 18 to 66 p.c., whilst our previous estimate corresponded to 42 p.c.

TABLE V. Nitrogen excreted by perfused hearts.

System	No. of hearts	Total wt. in g.	Urea N + NH_3—N (mg. per g. heart) (a) Control	(b) Perfusion fluid	(c) Difference	N excretion in mg. per c.c. of O_2 used	P.c. of total metabolism accounted for by N_2 excretion
Frogs' hearts.							
(a) Perfused Ringer's fluid							
(i) In air for 6 hr.	6	0·79	0	0·54	0·54	0·11	66
(ii) In oxygen for 6 hr.	12	1·33	0	0·59	0·59	0·11	66
(b) Perfused Ringer plasma mixture							
(i) In air for 6 hr.	4	0·47	0·75	1·02	0·27	0·04	24
(ii) In oxygen for 6 hr.	16	2·26	0·39	0·59	0·20	0·03	18
Tortoises' hearts.							
Perfused Ringer's fluid							
(i) In oxygen for 6 hr.	5	5·01	0	0·20	0·20	0·05	30
(ii) In oxygen for 24 hr.	5	5·01	0	0·42	0·42	0·04	24

FAT METABOLISM.

The fat content of the frog's heart was measured by the method of Stewart, Gaddie and Dunlop [1931] which estimated only the fatty acids, and this gave a value of about 1·5 p.c. fat. We previously [1931] estimated the fat content as 4·8 p.c., but the method employed [Stewart and White, 1925] included in the figure for fatty acids the phosphoric acid derived from phospholipins, and probably also some lactic acid formed from sugar during the alkaline hydrolysis.

TABLE VI. Fat metabolism of frogs' hearts perfused for 6 hours.

System	No. of hearts	Fat content of hearts: Average as p.c.	Fat content of hearts: σ_m of average	Fat removed from perfusion fluid (mg. per g. heart)
Unperfused controls	20	1·34	0·07	—
Perfused in oxygen				
Ringer's fluid	8	1·35	0·19	—
Ringer plasma	12	1·64	0·08	1·56
Ringer blood	12	1·70	0·09	—

We previously concluded that analyses failed to show any loss of fat after 24 hours' perfusion. Our present experiments also show no loss after 6 hours' perfusion. Indeed the experiments with plasma and blood actually show a gain in fat, and since this is about four times the standard deviation of either of the means compared, it appears to be a significant difference. Analyses of the fluids showed a slight loss of fat from the plasma perfused through the heart with no appreciable alteration in the total fatty acid content of the system heart + perfusion fluid.

The analyses therefore suggest that the heart takes up fat when this is present in the perfusion fluid, but they do not provide any support for the hypothesis that the hearts oxidize fats.

Partial desaturation of the fatty acids of the heart would account for some oxygen consumption, without the liberation of any CO_2 and without any apparent reduction in the fat content of the hearts. Table VII shows,

TABLE VII. Effect of perfusion on iodine number of fats in frogs' hearts.

System	No. of hearts	Total wt. in g.	Fat content p.c.	Iodine no.	Cholesterol p.c.
Unperfused controls	4	0·931	1·43	126	0·107
Perfused 6 hours with Ringer's fluid in oxygen	4	0·662	1·49	117	0·113

however, that perfusion caused no increase in the iodine number of the heart fats. Our figure for the iodine number (126) is higher than the results of Bloor [1926] who found in the ox heart an iodine number of 100 for the phospholipoids and 70 for the fats.

METABOLIC BALANCE SHEET.

The figures shown in our tables permit the construction of an approximate metabolic balance sheet in the case of frogs' hearts perfused in air and in oxygen with Ringer's fluid and with Ringer plasma. This is shown in Table VIII. The figures in the case of air are satisfactory, for the carbohydrate and nitrogen loss are adequate to explain practically the whole of the oxygen consumption and the calculated R.Q. approximates to the R.Q. found. The figures obtained with oxygen are, however, unsatisfactory because the carbohydrate and nitrogen loss together only account for about two-thirds of the oxygen consumption, and the theoretical R.Q. in both cases is higher than the value found experimentally.

An approximate balance could be attained for the two sets of figures obtained with oxygen by assuming that in each case the hearts used

TABLE VIII. Metabolic balance sheet for frogs' hearts.

(All quantities in mg. or c.c. per g. heart per 6 hours.)

	(i) Reducing substances		(ii) Nitrogen		(iii) Respiratory balance calc. from (i) and (ii)		(iv) Respiratory balance found by exp.	
	Loss	Oxygen equivalent	Loss	Oxygen equivalent	Oxygen used	R.Q.	Oxygen used	R.Q.
				Perfused Ringer's fluid				
(i) In air	1·30	0·97	0·54	3·2	4·2	0·85	4·8	0·90
(ii) In oxygen	0	0	0·59	3·5	3·5	0·80	5·4	0·73
				Perfused Ringer plasma				
(i) In air	7·82	5·8	0·27	1·6	7·4	0·98	7·08	0·91
(ii) In oxygen	5·20	3·9	0·20	1·2	5·1	0·96	8·10	0·76

The following factors were used in calculating these figures: 1 mg. $N_2 \equiv 6$ c.c. O_2 (R.Q. 0·8); 1 mg. glucose $\equiv 0{\cdot}75$ c.c. O_2 (R.Q. 1·0); 1 mg. fat $\equiv 2{\cdot}02$ c.c. O_2 (R.Q. 0·71).

about 1 mg. of fat ($\equiv$ 2 c.c. O_2) per g. Unfortunately the analyses shown in Table VI lend no support to this assumption. We are, therefore, forced to the assumption that in the case of a frog's heart working in pure oxygen about one-third of the oxygen is consumed by some process that does not involve the loss of carbohydrate or fat or involve the excretion of nitrogen, and that this oxygen consumption is associated with only a small excretion of CO_2.

DISCUSSION.

The authors commenced their investigations on the metabolism of the frog's heart in the expectation that the source of energy used by the heart would prove to be carbohydrate. Moreover the work of Clark and White [1928 *b*] had shown that any change that altered the mechanical response of the heart produced a parallel change in the oxygen consumption. The first facts established by our investigations were that the frog's heart perfused with Ringer's fluid and working in air used very little carbohydrate and excreted sufficient nitrogen to account for about half the oxygen consumption. Depression of the heart with lack of calcium or alcohol [1932 *a*] produced a greater proportional decrease in oxygen consumption than in nitrogenous excretion. The present experiments show that stimulation of the heart by perfusion with plasma increased the oxygen consumption, but reduced rather than increased the nitrogenous excretion. Perfusion of the heart with amino-acids produces a moderate increase in nitrogen excretion [1931]. Deprivation of oxygen reduced the nitrogen excretion of the heart to about one-

quarter [1932 *b*, Table V]. (This ratio, in the paper referred to, is stated on p. 329 to be 1 to 25, but this is due to an arithmetical error and the true value is 1 to 4.)

The nitrogenous metabolism of the frog's heart, therefore, remains remarkably constant as long as oxygen is present, but lack of oxygen causes a reduction in the nitrogenous metabolism and a compensatory increase in the carbohydrate metabolism occurs. Even partial oxygen lack causes the heart to convert carbohydrate to lactic acid. Not only does it act on its own carbohydrate, but will also convert glucose added to the perfusion fluid, although the heart when working in air makes no use of carbohydrate added to the perfusion fluid. Hearts in air use about 5 c.c. of oxygen per g. per 6 hours, a quantity which would be consumed by the oxidation of 7 mg. of carbohydrate, whilst the anaerobic heart can produce 34 mg. of lactic acid per g. per 6 hours [1932 *b*, Table V]. The relation between aerobic oxygen consumption and anaerobic lactic acid production in the heart shows, therefore, a considerable resemblance to the relation found in skeletal muscle; but the heart under aerobic conditions uses relatively little carbohydrate.

The substitution of pure oxygen for air does not produce any obvious change in the mechanical activity of the heart. The metabolism is, however, altered considerably. The nitrogen excretion is unchanged, but the carbohydrate metabolism is decreased and there is a remarkable fall in the R.Q., which can only be explained by assuming that about one-third of the oxygen used by the heart is utilized in some process with an R.Q. of 0·7 or even less.

It is surprising that the substitution of oxygen for air causes any change in the metabolism of the isolated frog's heart, because, according to the usually accepted formulae, air ought to provide a more than adequate supply of oxygen. The application of Warburg's formula shows that when a frog's heart in air consumes as much as 2 c.c. of oxygen per g. per hour, an adequate oxygen supply ought to be received when the sheets of muscle do not exceed a thickness of 0·25 mm.

We show in an appended note that the average thickness of the trabeculae of the frog's heart during diastole is about 0·05 mm. and about 0·1 mm. during systole. These figures indicate a large factor of safety, moreover the mechanical performance of the heart suspended in air appears normal and only traces of lactic acid are formed.

Pure oxygen certainly does not exert any toxic effect on the heart and our general impression has been that the hearts survive somewhat better in oxygen than in air. Our results suggest that reducing the

oxygen pressure causes changes in the metabolism before it causes any easily measurable changes in the mechanical activity of the heart. Our results show that in pure oxygen some substance with a low R.Q. is metabolized, but as regards the identity of this substance we can only say that there is no evidence of any fat loss. It is, however, possible that there is a fundamental fallacy in all our calculations regarding the metabolic balance sheet, since these have been based on the assumption that all oxidations in the isolated heart proceed to completion.

In the case of carbohydrate this assumption implies first that there is no appreciable accumulation of non-hexose reducing substances (such as methyl-glyoxal, pyruvic acid, etc.) and secondly that the "lactic acid" found does not include such substances. Case and Cook [1931] have shown that methyl-glyoxal is an intermediate in the breakdown of glucose to lactic acid by minced skeletal muscle of frogs, and hence the assumptions made are of doubtful validity.

In the case of fat there is the possibility that a shortening of the fatty acid chains occurs. Such a shortening would involve usage of oxygen with a very low R.Q. and would not lead to any reduction in the apparent amount of fatty acid present.

Smedley-MacLean and Pearce [1931] have shown that the oxidation of oleic acid involves splitting of the chain at the double bond, a process which actually leads to the production of two fatty acid molecules in place of one.

In view of these and other possible incomplete oxidations our failure to obtain a satisfactory balance sheet by the simplest possible interpretation of the R.Q.'s is not surprising, and we also feel that there are many possible explanations of the increase in reducing substances occasionally observed during perfusion.

Conclusions.

1. The following changes are observed when isolated frogs' hearts are kept in pure oxygen instead of in air:

(i) The R.Q. is greatly reduced.

(ii) The carbohydrate consumption is slightly reduced.

(iii) The total oxygen consumption is raised about 15 p.c.

2. We have failed to obtain any evidence that isolated frogs' hearts metabolize fat either in oxygen or in air.

3. In the case of hearts working in air, it is possible to work out a metabolic balance sheet, which is in approximate agreement with the measurements of the respiratory exchange and the heart analyses.

4. This cannot be done for hearts working in oxygen. In this case about one-third of the oxygen consumption appears to be utilized in some unknown process, which has a very low R.Q.

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A NOTE ON THE STRUCTURE OF THE FROG'S VENTRICLE.

The frog's ventricle is well known to be a sponge-like structure, but we know of no estimates of its internal surface. This was determined by the following method. Two frogs' ventricles were fixed with Bouinn's Fixative, one was in a contracted state and one was filled with fluid. Dr W. G. Millar kindly cut sections of these, from which micro-photographs were prepared. The length of the internal surface was measured by means of a map reader and the values obtained were reduced to the natural scale. The ventricles weighed 0·11 g. In the case of the contracted ventricle the total length of the internal surface shown was 3 cm. The heart was about 7 mm. long, and, if regarded as a cylinder 7 mm. long divided by vertical partitions, the total internal surface will be 21 sq. cm., or about 200 sq. cm. per g. of ventricle. Similar calculations in the case of the dilated ventricle gave a value of 320 sq. cm. per g. of ventricle. Direct observation showed that the average thickness of the trabeculae in the contracted and dilated hearts were 0·1 and 0·06 mm. respectively; this value agrees with the results of the calculations made

above. These figures are minimum values because the trabeculae were assumed to form sheets, whereas in reality they resemble rods more nearly than sheets.

The frog's heart is a much more favourable structure as regards thickness of tissue than even a sartorius of 50 mg., for the latter is about 0·5 mm. thick and has an area of only 40 sq. cm. per c.c. The ventricular muscle has five to ten times as great a surface per unit weight.

These calculations only apply, however, to a ventricle which contracts freely with good diastolic filling and thus irrigates its internal surfaces thoroughly. A strip of ventricle hung up in air has an effective surface of less than 30 sq. cm. per g., which is even lower than the sartorius.

612.328

THE GASTRIC FIBRES OF THE VAGUS NERVE.

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Ever since the work of Gaskell and Langley, the vagus nerve has occupied a firmly established position in the parasympathetic nervous system, and amongst its contributions to that system are included the vagal branches to the stomach. In conformity with the general arrangement of neurones in this system, the gastric branches of the vagus are usually considered to be long connector neurones arising in the bulbar nuclei and passing via the vagus trunk to Auerbach's plexus; hence a short effector neurone is relayed to the stomach (Fig. 1, A).

The bulbar roots of the vagus and its trunk above the two vagal ganglia consist almost entirely of medullated fibres, both small and large. Below the ganglia a marked difference is to be found in the composition of the nerve, owing to the appearance of a large number of non-medullated fibres; as the pharyngeal and laryngeal branches leave the parent trunk, they take with them the majority of the medullated fibres so that the proportion of non-medullated increases as the nerve enters the thorax, till finally its abdominal branches consist almost entirely of non-medullated fibres. These changes were recognized by Gaskell, Langley [Schafer, 1900], Ranson [1914] and others, but they made no suggestion that these non-medullated fibres were anything but vagal in their origin, although divergent views were expressed as to how the myelin sheath was lost. Recently Kiss and his colleagues have again drawn attention to this increase in the non-medullated components and have suggested a very different explanation of their presence and origin.

Kiss [1931] has made histological studies of the vagus in man, using an osmic acid technique. He states that non-medullated fibres are not present in the vagus till the lower level of the plexiform ganglion is reached, and he suggests that these fibres are really contributions from the superior cervical ganglion passing to the vagus via the lower anastomotic branches between the sympathetic and vagal ganglia. These anastomotic branches fall into two distinct groups, an upper and a lower; the upper group consists of fine medullated fibres, which Kiss compares

to the white ramus communicans of a spinal nerve; the lower group consists of non-medullated fibres comparable to the grey ramus communicans returning to the spinal nerve. It is, therefore, suggested that the vagus bears the same relationship to the sympathetic as does a typical spinal nerve—fine medullated connector neurones arise in the bulbar nuclei of the vagus, pass via the vagal roots, trunk, plexiform

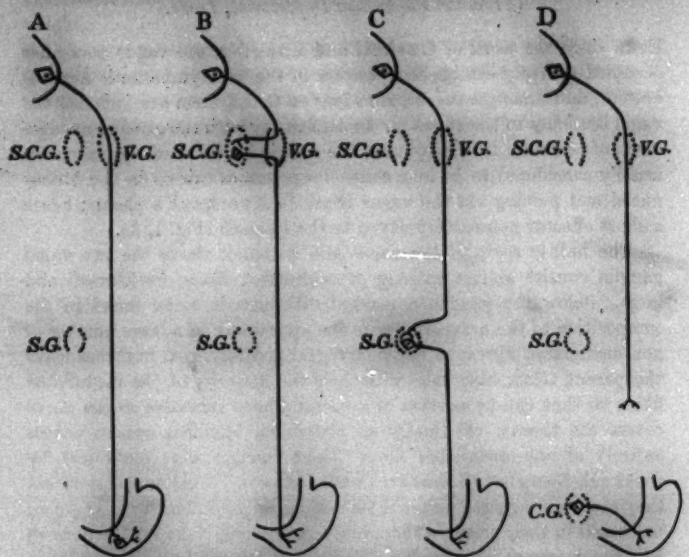

Fig. 1. The possible paths of the gastric fibres of the vagus. A, after Langley; B, C, D, after Kiss. *V.G.* ganglion trunci vagi; *S.C.G.* superior cervical ganglion; *S.G.* stellate ganglion; *C.G.* coeliac ganglion.

ganglion and upper anastomotic branches to a cell station in the superior cervical ganglion; in the superior cervical ganglion a second neurone begins, rejoins the vagus as a non-medullated fibre in the lower anastomotic branches and passes in the main trunk to its peripheral distribution. As the gastric branches of the vagus are almost entirely non-medullated, he therefore suggests that they have this sympathetic origin. Fig. 1 B gives a schematic representation of this suggested arrangement of neurones.

In further support of this idea, Kiss has made a series of comparative anatomical studies in which he claims that the innervation of the abdominal organs is always sympathetic in origin, though the path taken by the fibres varies in different species. Broadly speaking, the alternative paths fall into two groups, which are represented schematically in Fig. 1 (C, D)—in C the sympathetic contribution arises in the stellate or adjacent ganglia and passes to the thoracic vagus, whereas in D the sympathetic contribution passes direct to the stomach and the vagus, as such, does not reach that organ.

This conception of the arrangement of autonomic fibres in the vagus introduces a fundamentally new principle into the arrangement of the parasympathetic: Kiss goes so far as to say that in the abdomen no such separate system exists. Amongst his conclusions are the following statements: "The so-called parasympathetic influence of the vagus has no anatomical basis in the case of the abdomen. The parasympathetic phenomenon can be only a negative phase of the sympathetic."

Langley, Gad and Josephs [Schafer, 1900] considered that these vagal fibres lost their myelin sheath in their course, probably in the vicinity of the vagal ganglia, while Gaskell, from comparative studies, thought that these fibres represented a relay from a cell station on the vagus trunk.

From further histological studies Kiss [1932] states that he is able to identify a nerve cell which is "sympathetic," *i.e.* it gives rise to a non-myelinated fibre of the sympathetic system. These cells are found, not only in the sympathetic ganglia, but also in cranial and spinal ganglia, and they are therefore credited with supplying sympathetic elements within the cranial and spinal nerves. On these grounds he again makes the statement that: "There is no morphological foundation for the supposition of a cranial parasympathetic system."

Apparently Kiss takes the view that all the nervously controlled phenomena termed "parasympathetic" result from impulses passing down a system of non-myelinated fibres which are essentially "sympathetic" and arise from a certain type of cell within a sympathetic ganglion or in one of the cranial ganglia.

In order to test this theory we have performed experiments to determine the functional pathway whereby the vagus influences the stomach, and have tried to establish the source of the non-medullated components of the nerve. The animals employed have been cats. In these animals the histological differences in the vagus trunk are of a similar nature to those described in man—there are a few non-medullated

fibres in the vagal roots, but the number is markedly increased below the ganglion of the trunk. As the vagus does reach the stomach in the cat, condition D (Fig. 1) does not arise, and we have devoted our attention to the three other possibilities, A, B and C.

Effects of stimulation of intracranial vagus.

If the functional path of the gastric fibres is through the superior cervical ganglion, as suggested in B (Fig. 1), then removal of the superior cervical ganglion, and stimulation of the vagus above its ganglia should produce no effect upon the stomach. As the extracranial portion of the vagus above its ganglia is too short and inaccessible for satisfactory stimulation, we therefore proceeded to study the effect of stimulation of the intracranial portion of the vagus and the following technique was developed.

Method.

The animals were anæsthetized with ether, the carotids tied, and decerebration carried out by means of a trephine and scoop. The animals were then given a little more ether and the spinal cord was divided at the level of the axis vertebra; this left the animal with the lower part of the brain stem intact, but isolated from the spinal cord. All the operative procedures involved in the decerebration and spinalization were carried out with the cautery to minimize hæmorrhage. The animal was then allowed to recover for about 30 min.

The abdomen was opened and the pylorus was tied off; the œsophagus was opened in the neck and a stomach tube passed through the cardia and tied in position. The stomach was then washed out with saline and 60 c.c. of warm meat extract were introduced and the animal allowed a further 30 min. rest.

The cerebellum and bulb were now completely exposed by removing the overlying bone. The stump of the cervical cord was lifted up and gently mobilized until the bulbar roots of the vagus were clearly visible—the roots were easily found by following the spinal accessory nerve as it ran up along the lateral border of the bulb to join the lower vagal root. When the vagal roots were identified, two longitudinal cuts were made in the bulb, and its middle third removed—this allowed considerable freeing of the roots and avoided any risk of current spread to the opposite side. At this point, there was frequently troublesome oozing from the divided lateral sinus and bone, but this was controlled by light packing with wool. With this exposure it was possible to identify five bundles of

fibres going to form the vagus trunk—they could either be stimulated together or gently separated to permit of stimulation of individual bundles. There was no difficulty in identifying the spinal portion of the spinal accessory; it was much whiter and firmer than the vagal roots, which were pinkish, and soft in appearance, suggesting the absence of any quantity of supporting fibrous tissue.

Fig. 2. Gastric response to stimulation of the intracranial roots of the vagus. Cat 12. (1) Motor response—right vagal roots; coil 12, freq. 10: (2) inhibitor response—right vagal roots; coil 10, freq. 60: (3) motor response—cervical trunk of right vagus; coil 8, freq. 20. Time intervals 30 sec.

When the bulbar exposure was completed, the stomach-tube was connected to a recording manometer and the right carotid was cannulated. Stimulation of the intracranial vagus was then carried out either upon the whole nerve or upon individual roots. Towards the end of the

experiment the cervical vagus on the same side was stimulated for comparison of results.

When the results of intracranial stimulation had been established the same procedure was repeated in animals in which the superior cervical ganglion had been removed.

Experimental results.

Stimulation of the intracranial vagus produced exactly similar effects upon the stomach as did stimulation of the cervical trunk—motor or

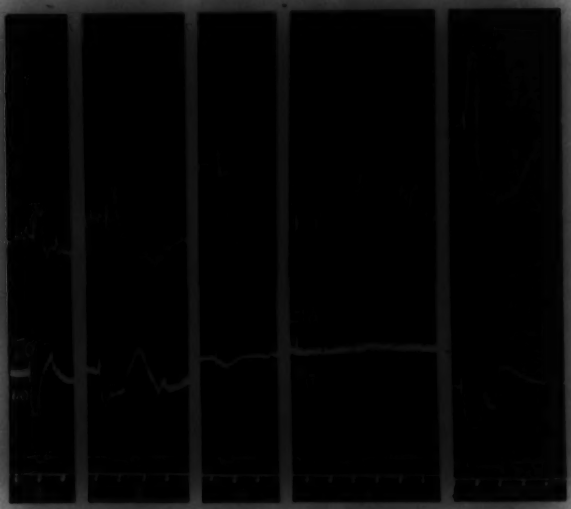

Fig. 3. Gastric response to stimulation of the intracranial roots of the right vagus after removal of the right superior cervical ganglion. (1–4) Stimulation of right vagal roots. Note the progressive diminution in the concomitant cardio-vascular response. (5) Stimulation of cervical trunk of right vagus after (4). Time intervals 30 sec.

inhibitor effects were recorded depending upon variations in the intensity of stimulation, tone of the stomach and the animal (Fig. 2). Selective stimulation of individual roots produced results which only varied quantitatively from those of the whole nerve, *i.e.* it was never possible to identify any particular bundle of fibres as being associated with any particular effect, motor or inhibitor.

The cardiac response to stimulation of the intracranial vagus showed very definite characteristics. At the commencement of the experiment even weak stimulation produced a marked and prolonged fall in blood pressure. As the experiment proceeded, the cardiac fibres became steadily less sensitive, till finally no cardiac effect could be elicited. That this loss of sensitivity of the cardiac fibres was purely a local condition was proved by the ease with which cardiac effects could be elicited via the cervical vagus long after the intracranial portion of the nerve was quite ineffective.

The disappearance of cardiac response did not affect the gastric response, showing that the latter was not dependent upon circulatory changes (Fig. 3).

The gastric response to stimulation of the intracranial roots of the vagus was quite unaffected by removal of the superior cervical ganglion (Fig. 3).

THE STELLATE GANGLION AND THE VAGUS.

Possibility C (Fig. 1) suggests that the gastric branches of the vagus might be derived from the stellate ganglion; if this were so, then removal of the stellate ganglion should render stimulation of the cervical portion of the vagus ineffective. Experiments were performed to test this point.

Method.

Decapitate cats were used. The stellate ganglion was exposed on one side and temporarily covered with a pad moistened with saline. The abdomen was then opened and the pylorus tied—a stomach tube was inserted through the œsophagus and connected to a water manometer. The right carotid was cannulated and the vagus nerve of the side corresponding to the exposed stellate ganglion was exposed and divided high up in the neck. Records of the gastric response to stimulation of the cervical vagus were then made over a wide range of intensities and rates. The stellate ganglion was then excised and a similar range of stimulation was repeated on the cervical trunk of the vagus.

Experimental results.

No difference could be detected between the gastric responses with and without the stellate ganglion.

THE ORIGIN OF THE NON-MYELINATED FIBRES OF THE VAGUS.

Having shown that the motor supply of non-medullated fibres to the stomach was not sympathetic in origin, it was necessary to enquire further into their mode of arrival. Two possibilities exist—they may

Fig. 4. Gastric response to stimulation of the cervical trunk of the vagus before and after removal of the corresponding stellate ganglion. Cat S 2. (1) Right vagus, ganglion intact; coil 4, freq. 24: (2) right vagus, ganglion removed; coil 4, freq. 24. Cat S 4. (3) Left vagus, ganglion intact; coil 3, freq. 60: (4) left vagus, ganglion removed; coil 3, freq. 60. Time intervals 30 sec.

represent connector neurones arising in the bulb and losing their medullary sheaths en route (Fig. 5, A), or they may represent a fresh neurone arising within one of the ganglia of the vagal trunk (Fig. 5, B). The former view was supported by Langley, Gad and Josephs [Schafer, 1900] but comparative studies suggested the latter explana-

tion to Gaskell. In the cat, longitudinal sections through the ganglion of the trunk show that the change to non-myelinated fibres takes place at that level.

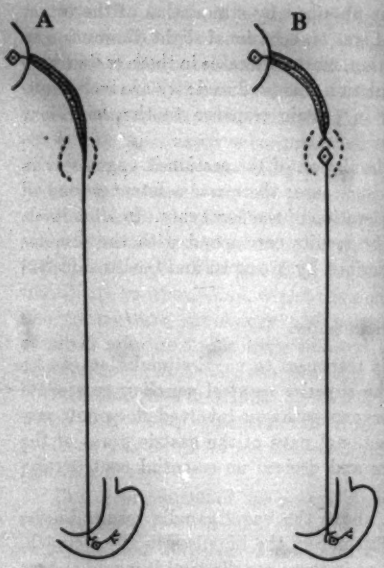

Fig. 5.

Fig. 6.

Fig. 5. Possible origins of the non-myelinated fibres. A, after Langley, Gad and Josephs; B, after Gaskell and Kiss.

Fig. 6. Effect of section and degeneration of vagus. Cat V 4. Right vagus divided above vagal ganglion 27 days previously. (1) Stimulation of cervical trunk of right vagus; coil 4, freq. 60: (2) stimulation of cervical trunk of left vagus; coil 4, freq. 60. Time intervals 30 sec.

Method.

In a series of cats the right vagus was divided immediately on emergence from the skull; the animals were allowed to recover and 10 to 28 days later the cervical vagus was stimulated and its effect on the stomach recorded.

Experimental results.

The cervical trunk of the vagus on the side of section was decidedly softer and more translucent than on the intact side. Stimulation of the sectioned vagus produced no motor or inhibitor effects upon the stomach in any way comparable to those obtained by stimulation of the intact nerve; the only change recorded was an occasional slight diminution in the amplitude of gastric contraction, most noticeable in those cases where a striking alteration in the circulation was produced: we are inclined to attribute this slight alteration in gastric response to the coincident vascular change.

The vascular response to stimulation of the sectioned vagus was in all cases a definite rise in blood-pressure; there was a latent period of 6 to 8 sec. and little, if any, acceleration of the heart rate. In all animals the right vagus was cut and the results correspond with the preponderance of pressor responses described by Morgan and Goland [1932] in the right vagus of the dog.

Discussion.

The fact that typical gastric responses to vagal stimulation can be obtained in cats deprived of the superior cervical ganglion or stellate ganglion indicates that the nervous pathway involved does not pass through these ganglia. The functional path of the gastric fibres of the vagus is confined to that nerve and derives no essential contribution from the sympathetic.

Section of the vagus trunk above the vagal ganglia results in the degeneration of the fibres which conduct the impulses to the stomach. Therefore no cell station is present in these ganglia, and it would appear that only one neurone is concerned in the conduction between the bulb and the stomach. Histological examination clearly indicates that the bulk of these fibres leave the medulla with medullary sheaths and reach the stomach as non-myelinated fibres; the site of loss of the myelin sheath is in the vicinity of the vagal ganglia.

As far as the efferent gastric fibres of the vagus are concerned, these experiments lend no support to Kiss's theories as to the relationship between the vagus and the sympathetic. Whatever may be the function of his "sympathetic" cells in the vagal ganglia, they are not the cells of origin of the non-myelinated efferent fibres to the stomach.

It has been suggested that the cervical sympathetic may send contributions via the superior cervical ganglion to the vagus, and that these

fibres are responsible for some of the gastric effects elicited by stimulation of the cervical vagus. These experiments show that stimulation of the intracranial roots of the vagus will produce the same range of gastric response as does the cervical vagus, a finding which disposes of any essential contributions from the cervical sympathetic.

Attention is drawn to the apparent difference between the cardiac and gastric fibres in the vagal roots. The cardiac branches are exceedingly sensitive at the commencement of the experiment, but this sensitivity rapidly diminishes till no cardiac effect can be elicited although the gastric response is undiminished (Fig. 3). This high degree of primary sensitivity with rapid subsequent fall suggests that the cardiac fibres have little protective covering in the vagal roots: thus they are readily accessible to stimulation but soon suffer damage thereby.

It has been suggested that the variations in gastric response to vagal stimulation are dependent upon or profoundly influenced by concomitant vascular changes. The fact that the intracranial cardiac roots become insensitive to stimulation, whilst the gastric fibres remain intact, enables one to separate circulatory and gastric effects; the response of the stomach appears to be little affected by the presence or absence of simultaneous circulatory changes (Fig. 3).

Stimulation of individual bundles of the vagal roots produced results which only varied quantitatively from those of the whole nerve. No evidence was obtained for the existence of special groups of motor or inhibitor fibres.

The arrangement of the gastric fibres of the vagus appear to conform to Langley's original conception of the parasympathetic system. A myelinated fibre leaves the bulb and passes via the vagus trunk to the stomach; in its course it loses its myelin sheath but has no cell station in the vagal ganglia.

Summary.

1. Stimulation of the bulbar roots of the vagus produces types of gastric response similar to those elicited from the cervical vagus.

2. Stimulation of individual roots produces no evidence of different types of nerve fibre responsible for motor or inhibitor effects on the stomach.

3. Vascular changes are not responsible for the various types of gastric response.

4. The functional path of the gastric fibres of the vagus is confined to that nerve, and has no essential connection with the sympathetic.

5. There is no cell station for the gastric fibres in the vagal ganglia. Myelinated fibres leave the bulb and pass directly to the stomach, losing their myelin sheaths en route.

One of us, W. R. Spurrell, is indebted to the Medical Research Council for a personal grant.

The expenses of this investigation have been defrayed in part by a grant from the Government Grant Committee of the Royal Society.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

October 22, 1932.

The fallacies of indirect calorimetry. By T. W. Adams and E. P. Poulton.

Insulin raises the R.Q. in depancreatized animals and so increases the utilization of carbohydrate; but in depancreatized animals the R.Q. may be fairly high—up to about 0·80, so that the power to utilize carbohydrate is not lost. These facts, apparently contradictory, led us to question the basis of indirect calorimetry, viz. that the level of the non-protein R.Q. indicates the proportion of C. to F. oxidized, the value being based solely on the chemical composition and the heat combustion of C. and F. [Zuntz and Schumburg]. Those published experiments were examined in which the calories, O_2 and CO_2, were simultaneously determined over periods of 1, 2 and 3 hours. In each case non-protein values were obtained and the proportions between indirect and direct calorimetry calculated; these quantities were then plotted against the R.Q. In the figures the heat measured is taken as 100 p.c. and is represented by the horizontal line at zero; the ordinates for the top part of the diagram represent the percentage differences between the calculated and measured heats. The proof that it is permissible to use the Zuntz-Schumburg figures will depend on the black dots lying evenly distributed above and below the horizontal line throughout the range of R.Q.s. This is obviously not the case (Figs. 1, 2 and 3), and a fall in the percentage differences as the quotient rises has been found in all the published experiments on fasting subjects.

There is a slight difference in the slope of the results for A. H. M. and A. L. L., and the mean slope for these two men is the same as that drawn in Fig. 3 and it cuts the zero line at R.Q. 0·785, indicating a combustion of C. to F. in the ratio of 1 : 1·36. Hence though it is necessary to give up Zuntz's theory the provisional hypothesis may be advanced that C. and F. are always burnt in this constant proportion and at other R.Q.s additional metabolic changes take place which influence the heat produced. In this connection it is interesting that when the fast is prolonged the point of intersection of the slope with the zero line shifts to the left (Fig. 3) indicating that the proportion of C. to F. burnt has changed for S. A. B. in the course of a 3 days' fast from 1 : 1·14 to 1 : 3·34; this is perfectly

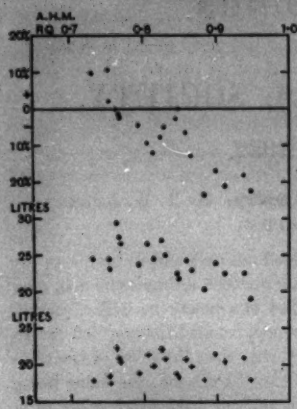

Fig. 1.

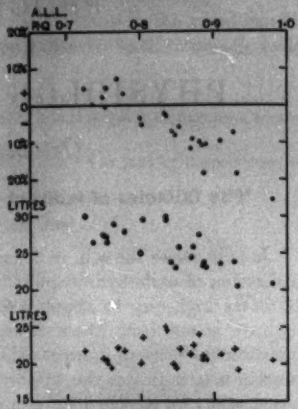

Fig. 2.

Comparison of indirect and direct calorimetry; o oxygen; + CO_2, two hourly determinations during first day of fasting. Subject at rest. *Carnegie Institution Publ.* 261.

Figs. 1 and 2. Periods of 2 hours each during day.

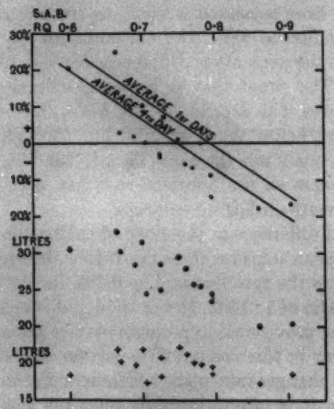

Fig. 3.

Subject not at complete rest. *Carnegie Institution Publ.* 77.

Fig. 3. Periods of 2 hours each, from 9 a.m.—11 p.m.

intelligible considering that there is less C. now available in the body. Fortunately in most determinations of the basal metabolism the R.Q. is in the neighbourhood of 0·80 so that the Zuntz-Schumburg figures usually give correct results.

The thermochemical equations for the conversion of C. to F. without the formation of intermediate products do not account for the difference between the calculated and measured heats, and so it is probable that temporary partial metabolic changes are taking place.

In the figures the O_2 and CO_2 values have also been entered, and it is of particular interest to note that while the CO_2 remains constant the O_2 intake diminishes as the R.Q. increases. This is the converse of what happens when sugar is taken.

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On the mode of action of vasomotor nerves. By W. A. Bain.
(*Department of Physiology, University of Edinburgh.*)

That certain nerves produce their effects by the production or liberation at their endings of specific chemical substances is now fairly well established. This has been directly demonstrated by Loewi [1921] and numerous others in the case of the cardiac nerves of the frog, by Rijlant [1927] for the cardiac nerves of mammals, by Finkleman [1930] and by Cannon and Bacq [1931] for the sympathetic supply to mammalian plain muscle. Indirect proof that vasodilator nerves act likewise has recently been given by Dale and Gaddum [1930].

An attempt has been made to obtain direct evidence in favour of the view that vasomotor nerves act by the production or liberation of specific substances at their terminations. In these experiments the tongue (*in situ*) of the dog, perfused with Dale's solution by means of a Dale-Schuster pump, was used. The fluid entered the tongue *via* the lingual artery and on leaving *via* the vein was immediately led to an intestine bath in which was suspended a short piece of rabbit intestine as a test tissue. The perfusion pressure was recorded by a mercury manometer connected with the output tube of the pump. In order to ascertain whether the rate of flow through the tongue varied with changes in calibre of the tongue vessels, the outflow from the tongue was recorded by an electric tilter operating from the overflow of the intestine bath. By this means it was easy to show that the effects obtained on the isolated

intestine by stimulation of the vasomotor nerves of the tongue were not due to changes of temperature in the intestine bath consequent upon a changed rate of flow through the tongue vessels.

It has been shown that: (1) stimulation of the peripheral end of the lingual nerve confers upon the perfusing fluid properties which render it excitatory to an isolated length of rabbit intestine, causing an increase in amplitude of the contractions and usually also an increase in tone of the muscle. The average increase in amplitude over the normal varies in different experiments between 20 and 80 p.c.; (2) stimulation of the peripheral end of the vagosympathetic trunk confers upon the perfusing fluid properties which render it inhibitory to an isolated length of rabbit intestine, the contractions becoming diminished in amplitude and the tone usually becoming slightly reduced. The average decrease in amplitude below normal varies between about 20 and 40 p.c. in different experiments.

It is concluded that these effects on the isolated intestine strip are due to the humoral transmission of specific substances produced or liberated by the agency of the nerves supplying the tongue vessels, and that it is by the production of these substances and their subsequent action upon the muscular tissue of the vessels that the vasomotor nerves of the tongue produce their effects. The fact that definitely positive results have not been obtained in cases where blood was present in the perfusing fluid supports the view that, under normal circumstances, the neuromimetic substances are either not passed into, or are rapidly destroyed by the blood. It is therefore suggested that normally these neuromimetic substances produce their effect locally, at the site of production, and that any humoral transmission to other sites is probably incidental and unimportant.

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The action of œstrin on the isolated uterus. By G. F. Marrian and W. H. Newton.

For these experiments, strips of uterine muscle have been taken from guinea-pigs and suspended in the solution recommended by Burn and Dale [1922]. To the solution have been added oxytocin and pure crystalline œstrin. Both the trihydroxy- and ketohydroxy- varieties of

the latter have been used, dissolved in either absolute alcohol or sodium hydroxide solution. When alkali has been employed, an equivalent amount of hydrochloric acid has been added simultaneously, and in all cases control experiments have been performed, the solvent being added without the œstrin. 5 p.c. carbon dioxide in oxygen has been bubbled through the bath instead of pure oxygen, so as to complete the bicarbonate buffer system.

Fig. 1 shows eight consecutive responses of a virgin uterus to the same dose of oxytocin (0·025 unit), the solution being changed after each contraction and the interval between each being the same (15 minutes). Before the first two doses there was no preliminary treatment; 2½ minutes

Fig. 1. Virgin guinea-pig, 220 gm. Eight consecutive doses of 0·025 unit oxytocin at 15 min. intervals.

before each of those of the second and fourth pair, 0·1 c.c. of absolute alcohol was added to the bath; 2½ minutes before each of the third pair, was added 0·21 mg. of ketohydroxy-œstrin dissolved in 0·1 c.c. of absolute alcohol.

The same result has been obtained for both the trihydroxy- and ketohydroxy- compounds, using for the test, instead of the virgin uterus, the uterus three months after oöphorectomy.

The second half of the tracing in Fig. 2 shows the reaction of the pregnant uterus to oxytocin (0·025 unit, left in the bath), and the subsequent effect of 0·2 mg. of ketohydroxy-œstrin dissolved in sodium hydroxide solution, the equivalent of acid being added simultaneously. The first half of the tracing shows the effect of equal amounts of alkali and acid under the same conditions. Exactly the same effect is seen when alcohol is used as a solvent; it may be summarized as a more rapid relaxation of the strip from each contraction, a return to a much greater resting length, and a speeding up of the rhythm.

One conclusion may be drawn from the combined results of the three sets of experiments (virgin uterus, involuted uterus and pregnant uterus), namely, that œstrin counteracts the tendency of oxytocin to produce any sort of sustained contraction of the uterus. Its effect on rhythmic contraction is not so clear. Fig. 2 would suggest that the combination of oxytocin with œstrin is ideal for labour, and this may be so. But the

Fig. 2. Pregnant guinea-pig, 600 gm. Nearly at full term; length of fœtus 10 cm. *OX* ≡ 0·025 unit oxytocin. The two halves of the tracing are continuous.

mature non-pregnant uterus, and the uterus of early pregnancy, show maximal rhythmic contractions in the absence of oxytocin, and the action of œstrin upon these is depressor both as regards their height and frequency. The possibility must be considered, therefore, that the heightened rhythm in Fig. 2 is in some way due to the more relaxed state of the muscle rather than to the presence of œstrin.[1] We have been handicapped throughout by the insolubility of œstrin in physiologically inert solvents, and the consequent impossibility of adding more than a small quantity to the bath (capacity 60 c.c.).

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Gas tensions in the gall bladder. By J. Argyll Campbell.
(*National Institute for Medical Research.*)

There are no data, so far as I know, regarding the normal carbon dioxide and oxygen tensions in the gall bladder. Previously attempts were made to estimate the tensions in bile by estimations of the volume

[1] Confirmed later.

p.c. of gases removable by gas pump from bile. The literature on this point was reviewed recently [Campbell, 1931], and no definite conclusions could be drawn.

In the present research the animals were anæsthetized (A.C.E. and amytal), the abdomen opened and a gas mixture, containing oxygen, carbon dioxide and nitrogen in varying tensions (see Table I), was injected carefully into the gall bladder in its normal condition and containing bile. Sufficient gas was injected to distend the bladder moderately, a fine hypodermic needle and rubber bag being employed. No ligatures were applied to the ducts, etc., but the abdomen was closed. The gas was left in the gall bladder for various periods, and then samples were withdrawn carefully by means of a similar needle and a gas sample tube or 1 c.c. syringe and then analysed by the modified Krogh method [Campbell, 1932 *a*] or by the Haldane method. The former method was employed when only a bubble (up to 0·15 c.c.) could be obtained for analysis; the Haldane method was used when 1 c.c. or more of gas was available for analysis, the gas being then mixed with a known amount of nitrogen in the 10 c.c. apparatus.

From the details shown in Table I and after allowing for the effects of

TABLE I. Gas tensions in gall bladder.

Date	Animal	Tensions mm. Hg in gas injected CO_2	Tensions mm. Hg in gas injected O_2	Tensions mm. Hg in gall bladder CO_2	Tensions mm. Hg in gall bladder O_2	Time gas was left in gall bladder (hours)	Method of gas analysis
30. vi. 32	Rabbit	0	43	47	42	2·50	Krogh, modified
				49	40	3·75	
				56	45	4·75	
6. vii. 32	,,	0	7	49	42	1·50	,,
13. vii. 32	,,	0	19	63	18	3·00	,,
15. vii. 32	,,	0	37	38	29	4·00	,,
19. vii. 32	,,	0	4	38	28	4·00	,,
20. vii. 32	,,	0	5	31	27	3·00	,,
				36	32	4·50	
5. vii. 32	Cat	0	4	35	28	2·00	,,
				41	33	3·00	
28. vii. 32	,,	40	26	45	45	3·00	Haldane
28. ix. 32	,,	0	4	35	28	1·66	,,
				56	19	4·00	
29. ix. 32	,,	0	4	53	7	2·00	,,
				40	17	3·00	
30. ix. 32	Dog	0	4	42	7	1·50	,,
				47	13	3·83	
				48	11	5·50	

anæsthetics [Campbell, 1932 *b*] it is concluded that the normal carbon dioxide tension in the gall bladder of the rabbit, cat and dog varies between 35 and 60 mm. Hg, and the oxygen tension between 17 and 45 mm. Hg. There is a suspicion that when the oxygen tensions fell below

20 mm. Hg, the circulation in the liver was being interfered with by the position of the animals, which were tied down on their backs. The circulation in these cases did not appear to be active either in the vessels of the stomach or in those of the liver. The supine position is not a normal position for these animals. Nevertheless, there is a general agreement between these results and those published for gas tensions in other tissues of the body [Campbell, 1931, 1932 *b*, *c*]. Many of the latter results were obtained from animals in the normal prone position.

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Hypertrophy of the heart in acclimatization to chronic carbon monoxide poisoning. By J. Argyll Campbell. (*National Institute for Medical Research.*)

In the course of the post-mortem examinations of tar-cancer mice, some of which had been exposed for about 9 months to chronic carbon monoxide poisoning and others for the same period to oxygen at high pressure (60 p.c. atmos.) in the air, it was observed that the average weight of the hearts of the mice exposed to carbon monoxide was much greater than that of the mice exposed to high oxygen and also than that of the control mice (see Table I). Only one of the controls and two of the mice exposed to high oxygen possessed hearts weighing more than 0·235 gm. Of the 19 mice exposed to carbon monoxide, 14 possessed hearts weighing between 0·235 and 0·340 g.; in 4 others the weights were between 0·200 and 0·235 g.; 0·435 g. was the weight of the heart in the remaining mouse. The mice were gradually acclimatized to the carbon

Table I. Hearts of all the mice surviving to the end of experiment.

Condition of mice	No. of mice	Heart weights (g.)	
		Average	Limits
Exposed to CO	19	0·277	0·435 – 0·200
Exposed to high O_2	33	0·182	0·260 – 0·110
Controls	32	0·165	0·260 – 0·118

monoxide, so that on the 1st, 5th, 10th, 20th, 24th, 27th, 39th and 46th days the percentages of carbon monoxide in the air breathed were 0·04, 0·06, 0·09, 0·10, 0·13, 0·17, 0·19 and 0·24 respectively. From the 46th day till the 269th day or end of the experiment the carbon monoxide was

maintained at 0·24 p.c. and the surviving mice were evidently acclimatized to this amount as their body weights were as well maintained as those of the other two groups. The respiration chambers employed have been described elsewhere [Campbell, 1932].

The carbon monoxide, on the whole, retarded the development of tar warts and cancer, and this aspect of the experiment will be considered in another paper. Taking ten mice of each group which were equally affected by the tar—judging from the extent of the scar formation, etc.—we have the following weights for the hearts at the end of the experiment (Table II).

TABLE II. Hearts of ten tarred mice of each group compared with those of ten normal mice of similar body weight (28 g.).

Condition of mice	Heart weights (g.)	
	Average	Limits
Exposed to CO	0·282	0·435 – 0·210
Exposed to high O_2	0·195	0·260 – 0·150
Tarred controls	0·153	0·190 – 0·130
Normal controls	0·149	0·170 – 0·130

We have also data from a few untarred mice which were included in the same long experiment; weights of the hearts are given in Table III, the body weights (28 g.) and diet of each group being similar.

TABLE III.

Condition of mice	No. of mice	Heart weights (g.)	
		Average	Limits
Exposed to CO	2	0·260	—
Exposed to high O_2	4	0·177	0·190 – 0·165
Controls	4	0·182	0·207 – 0·155

In all cases the entire hearts were weighed moist immediately after the animals were killed, the cavities being slit open to remove blood and blood clots.

The hypertrophy of the heart muscle probably follows the increase in viscosity of the blood owing to increase in red corpuscles [Naismith and Graham, 1906; Campbell, 1929, 1932].

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An isolated mammalian heart preparation capable of performing work for prolonged periods. By I. DE BURGH DALY and W. V. THORPE. (*Preliminary communication.*)

It has been reported [Daly, 1927; Verney, 1927] that an isolated dog's heart preparation performing work rapidly becomes hypodynamic in spite of good oxygenation of the defibrinated blood by some form of Hooker-Drinker apparatus. The onset of cardiac failure generally occurs within 20–60 minutes and is in marked contrast to the conditions found in the heart-lung preparation which, if not overworked, may continue to function well for 5 hours.

The following were considered possible causes of the early cardiac failure in the isolated heart preparation: (1) the removal of any nervous control arising in the lungs; (2) damage to the heart due to operative interference; (3) loss of water from the blood due to evaporation in the oxygenator; (4) physico-chemical changes in the blood due to its passage through the oxygenator with the formation of substances causing the cardiac failure; (5) the addition to or removal from the blood by the lungs of one or more substances necessary for the proper functioning of the heart. We have shown that the factors (1) to (3) are not responsible for the early cardiac failure, although it is true that the heart lasts longer if the pericardium is not opened except for a small slit opposite to the left auricular appendage for insertion of the cannula. Our experiments indicate that an essential condition is extensive blood filtration before and during the experiment through finer cambric filters ($50 \times 100\mu$ mesh measured dry) than those usually employed for heart-lung preparations ($100 \times 200\mu$). The blood should be filtered through four or five filters immediately after defibrination and just before use, and two filters kept in the circuit during the experiment. Anrep and Häusler [1928] stress the importance of thorough blood filtration and detoxication in their experiments on the perfusion of a coronary artery in the heart-lung preparation. We are able to confirm the importance of these factors relative to the isolated heart doing work and the perfused heart performing no work; moreover, we gain the impression that circulation of all the blood through the heart-lung preparation before switching over to the isolated heart, largely prevents the clogging of the filters during the experiment. With these modifications of the usual technique we have been able to keep the isolated heart of a dog performing work over 5 hours (pericardium closed) in addition to 1 hour during which the heart-lung preparation was running. It is clear that the isolated heart requires more perfect filtration of the blood than the heart-lung preparation, and the

reason may be that the rôle of the lungs as a mechanical filter of blood is lacking, or that changes in the physico-chemical state of the blood in the absence of the lungs and in the presence of the oxygenator may lead to the formation of particles injurious to the heart.

The problem of the oxygenation of the blood was solved by building an apparatus which is essentially three Hooker-Drinker oxygenators arranged concentrically. This apparatus, which has a surface area of 1·1 sq. m., can maintain blood at 90–100 p.c. saturation during the experiment.

In two experiments the oxygen consumption per g. of heart per hour varied between 1·4 and 5·6 c.c. These figures agree fairly well with those of Lovatt Evans [1912] who obtained corresponding values ranging from 3·5 to 6·0 c.c. For comparison of the oxygen consumption of the heart at different stages in the experiment, we have estimated the oxygen consumption per g. of heart per kg. work per hour; this value is found to increase as the experiment progresses.

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A simple method of appreciating the muscular sense.

By D. F. Fraser-Harris.

One lies supine full-length in a large bath of water. Allow one leg, abducted and slightly flexed at the knee, to float comfortably on the surface of the water. Note the minimal muscular sensations from this resting limb (physiological zero).

Now very slowly begin to immerse the leg, still abducted and flexed, and note the sense of effort (resistance) due to the resistance of the water.

Let the leg return to the surface and once more float at rest, when one perceives a distinct and immediate diminution of the sense of effort.

Finally keeping the leg rigid as one segment from the hip joint and still abducted and flexed, raise it slowly into the air against gravity.

Note the very marked increase of muscular sensation (sensation of weight) which is maximal at the moment of the limb leaving the water and becomes less and less massive as the vertical is approached.

Exactly similar but less massive muscular sensations may be experienced by lowering and raising the arm in one segment from the shoulder joint.

This method, requiring no apparatus, is more "natural" than that of faradizing the muscles (say) of the arm through the skin. In the method just described, the muscular sensations are not complicated by the unpleasant pricking cutaneous sensations produced by the faradic currents traversing the skin.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY,
November 19, 1932.

The rate of glomerular filtration in the perfused kidney.

By A. Hemingway. (*Physiology Institute, Cardiff.*)

The amount and rate of glomerular filtration occurring in the kidney of the pump-lung-kidney preparation has been studied according to the creatinine elimination method of Rehberg [1926].

Creatinine is added to the perfusing blood in the proportion of 10 mg. of creatinine dissolved in 1 c.c. of saline for each 100 c.c. of blood. After allowing time for thorough mixing, simultaneous readings are made of blood plasma creatinine concentration (C_b), urine creatinine concentration (C_u), rate of urine output and rate of renal blood flow.

The concentration ratio of creatinine between blood and urine $\left(\frac{C_u}{C_b}\right)$ is calculated, and, multiplied by the rate of urine formation (U), gives the rate of glomerular filtration (F). The amount of fluid reabsorbed in the tubules (R) $= F - U$.

Table I. Rate of glomerular filtration in the perfused kidney.

Exp.	Wt. of kidney g.	U	C_u mg./100 c.c.	C_b mg./100 c.c.	F	R	$\frac{R}{F} \times 100$	Blood flow
1	23·4	0·74	130·0	16·30	5·92	5·18	87	—
2	22·0	0·72	14·3	3·08	3·31	2·59	76	134
3	24·4	0·90	59·0	4·20	12·60	11·70	93	116
4	32·2	1·06	42·0	6·25	7·10	6·00	85	71

All rates of flow are given in c.c./min.

Table I shows the rates which have been measured in some preliminary experiments. The maximum rate of glomerular filtration has not been greater than one-tenth of the blood flow, and the amount of fluid reabsorbed has not been less than 75 p.c. of that filtered through the glomerulus.

During an experiment it has been noticed that, while the rate of urine formation may increase, the rate of formation of glomerular filtrate diminishes, but the proportion of it which is reabsorbed is smaller.

It is intended in further experiments to compare the rate of elimination of creatinine with that of some non-metabolized sugars.

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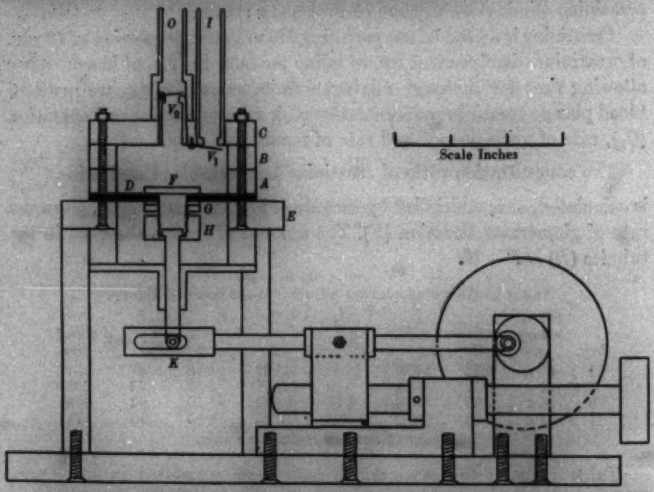
A new perfusion pump. By A. Hemingway.

(*Physiology Institute, Cardiff.*)

The introduction of erinoid[1] has made possible the construction of a perfusion pump having a solid cylinder which does not expose blood to a metal surface.

The design of the pump, which is of the rubber diaphragm type, is similar in its principles to that introduced by Dale and Schuster [1928], but obviates the use of a glass blood chamber from which the blood is propelled by the movements of a rubber finger stall.



The pump cylinder is built up of two rings (*A* and *B*) of $\frac{1}{4}$ in. erinoid covered by a top plate *C*. The rings are clamped together and held to a base ring by bolts passing through their thickness. The top plate carries the inlet and outlet tubes (*I* and *O*) and their valves (V_1 and V_2). The tubes and valve seatings are made in erinoid, the valve flaps are cut from thin sheet rubber and secured to the valve seating by 14 B.A. screws. The heads of the screws are covered with celluloid varnish.

The circular diaphragm, *D*, is cut from sheet rubber $\frac{1}{8}$ in. thick and is firmly clamped round its periphery between the erinoid ring *A* and the

[1] Erinoid is a coagulated casein product and may be obtained in sheets or rods from Erinoid Ltd., Stroud, Gloucester.

metal ring *E*. Connection is made to the piston rod through an erinoid plug *F*, which is clamped to the rubber by the screwed ring *G*, and to the driving rod by the flanged fitting *H*.

The method of varying the stroke while running is by alteration of the fulcrum which works in a slide. The thrust from the crank to the piston rod is transmitted through the fork *K* and a roller bearing.

Direct comparison of this pump with a glass-and-rubber pump of the finger-stall type [Hemingway, 1931] was made by changing from one to the other during an isolated kidney perfusion. There was no change in the blood flow through the perfused organ when the change-over was made.

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A one screw-fixing boss head. By A. Hemingway and A. L. Sims.
(*Physiology Institute, Cardiff.*)

The boss head described has the advantage that it can be moved to any position and both rods held in it clamped with great rigidity and at right angles by tightening only one screw.

The method of construction is shown in the diagram. Two similar rectangular blocks of brass, 2 in. × ½ in. × ½ in., have a ⅜ in. diam. hole drilled with centre ⅝ in. from one end. A V-groove is cut in the middle of the inner face at right angles to the hole. Beyond the groove, holes are drilled to take the clamping screw, and the faces are cut away so that the blocks can be held at an angle to each other when clamping small rods.

Modification of speech sounds in passing through acoustic filters. (Demonstration.) By F. H. Gage and J. H. Shaxby.

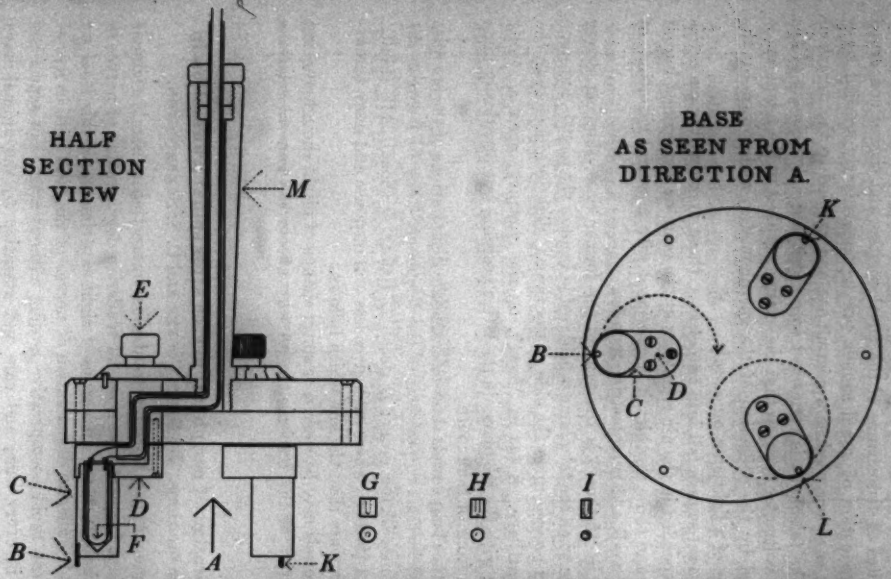
The type of hearing associated with high-note deafness can be roughly imitated by the sounds transmitted by a speaking tube in which is included a low-pass acoustic filter. The high notes associated with consonants and, to a less extent, with most of the vowels are by this means greatly weakened, and the resulting speech sounds, though audible, are so distorted and their characteristic differences so much reduced that they are only with difficulty intelligible.

The acoustic filter used in the demonstration consists of a brass tube forming part of the conduit through which the sounds pass, pierced with a series of rings of holes, each ring opening into a separate short cylindrical chamber, concentric with the central brass tube.

The dimensions of these cylinders are such that notes higher than about 800 ~ are cut off, lower tones being transmitted with very little loss, while those above the cut-off lose twenty or more decibels in passage through the filter.

A new differential æsthesiometer. By T. Graham Brown.

The object of this instrument is to test the possibility of the sensory localization of a heat stimulus as such. Three similar metal discs (the surface areas of which may be varied in different experiments—discs of diameters between 1·5 mm. and 6·0 mm. are used) are so arranged that they may be placed equi-distantly, and at any selected distance between 6 mm. and 80 mm. apart. One of the three discs contains a resistance wire which is capable of heating it to different temperatures by the passage of an electric current. There is no outward indication on the instrument of the disc which may be so heated. The three discs are placed on the skin of a subject at a selected distance apart—one disc having been heated to a desired temperature. As all other stimuli (such as pressure and touch stimuli) are common to the three discs, if the subject experiences a heat sensation, and can localize it at the area of skin touched by the heated disc, he then localizes a sensation of heat as such. The discs are capable of being arranged not only in an equi-distant pattern, but also in any other which falls within the area of a circle the diameter of which is about 90 mm.



Differential æsthesiometer. *B*, rod (provided with screw) forming smallest contact disc and inserted in margin of metal cylinder. *C*, metal cylinder, which is capable of rotation round point *D* by means of milled head *E*. *F*, resistance wire enclosed in cylinder *C*. *G*, *H* and *I*, discs of different diameters capable of being screwed on to *B*, *K* and *L*. *K*, and *L*, contact discs similar to *B*, but not capable of being heated. *M*, handle of instrument containing electric leads to resistance wire in *C*.

The regions of greatest energy in the production of vowel sounds. (Demonstration.) By F. H. Gage and J. H. Shaxby.

The sites of disturbance of the vocal cavities and tissues concerned in the production of vowel sounds can be conveniently studied by a telephone ear-piece used as a microphone and applied to the mouth or throat. The telephone is connected to a rectifying valve circuit of the Moullin voltmeter type, so that the energy supplied to the telephone is manifested by the deflection of the galvanometer, and shown by the movement of a light-spot on a scale.

The vowel sounds fall into two classes, those in which the energy is mainly expended in the air vibrations at the more or less opened buccal aperture, and those in which it is associated with powerful vibrations of the vocal cords and neighbouring muscles.

A (as in "father") is the type of the former class, showing strong deflections when the telephone is held just in front of the mouth, but none when it is pressed upon the side of the throat, slightly higher than the thyroid cartilage. *E* (as in "feet"), on the contrary, gives a strong deflection with the telephone in the latter position, but practically none with it in front of the mouth.

The other long vowels show intermediate distributions of the energies manifested in the two positions of the telephone. They lie between the two extremes in the order: *E* (feet), *Ů* (boot), *O* (boat), *AW* (fault), *Ê* (fate), *A* (father), where each gives less oral response and more throat-muscle response than the one which follows.

The vowels may be spoken or sung; change of pitch, though altering the intensity of the response, in no way affects the general relations described.

Degeneration of peripheral nerves after spinal transection in the monkey. By Sybil Cooper and C. S. Sherrington.

In the monkey (*e.g.* Macaque) total spinal transection in the mid-thoracic region brings frequently as a sequel Wallerian degeneration of nerves in the hind limb (1), (2), (3). In a series of eight experiments of this kind we have found Wallerian degeneration of nerves of the hind limb in five of them. Clean rapid postoperative healing without a trace of sepsis has occurred in every case, and care and attention to the animal have avoided the incidence of bedsores. There has been no complication by myelitis; therefore the possibility of myelitic degeneration of the motor horn cells as accounting for Wallerian changes in the fibres in the limb

nerves is excluded. A feature presented by the peripheral degeneration is that in some cases in certain of the affected nerve trunks not a single myelinated fibre remains sound. In our experience the degeneration is always confined to the sciatic nerve and its branches. A character observed has been the striking asymmetry in the amount and distribution of the degeneration in the nerve trunks of the right and left limbs. Thus the degeneration may be very slight in certain nerves of one limb and entirely destructive of those same nerves in the other limb.

In three only of these experiments have the spinal nerve roots of the hind limb, as well as the limb nerves themselves, been examined for Wallerian degeneration. The search revealed no trace of degeneration in either the ventral or dorsal roots, although the degeneration in nerves of the limb was very heavy. In these three experiments the periods allowed were four, seven and two weeks respectively. Notwithstanding absence of degeneration in the spinal roots, the sciatic nerve trunk was heavily degenerated even as far proximal as the level of the hip joint.

An inference to be drawn is that some traumatic damage is likely to supervene in the sciatic nerve in the monkey in a paraplegic state, although the animal be provided with soft hay bedding in a roomy cage.

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PROCEEDINGS

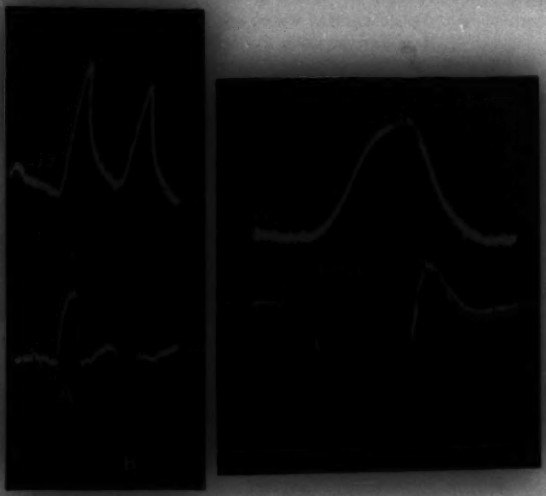
OF THE

PHYSIOLOGICAL SOCIETY,

December 17, 1932.

A convenient method of studying the reactions of the vasomotor centre. By R. J. S. McDowall. (*Dept. of Physiology, King's College, London.*)

The method consists of a combination of that used by Bartlett and that demonstrated by me to the Society in 1923 and subsequently described by Hemingway. It consists essentially of recording the perfusion resistance of a part of the body, the blood vessels of which



have been tied off for the most part from the general circulation. The apparatus can be made to give magnification several times that of the blood-pressure variations, and all the ordinary reactions of the vasomotor centre may be shown with complete readiness and uncomplicated by the cardiac or peripheral vascular effect. The figures indicate typical

records obtained by the method. The upper tracing in each instance indicates perfusion resistance in the hind limbs of a cat, the lower, blood-pressure in the left carotid artery, the vagi being cut in each case. At *A* the right carotid artery was clamped, *i.e.* the carotid sinus clipped off, temporarily between the arrows, at *B* the heart was held. These records show the reaction of the centre to fall of arterial pressure. In *C* the animal was asphyxiated between the arrows. The stimulation of the vaso-motor centre due to the asphyxia is seen in the perfusion record, although it is completely masked in the record of arterial pressure. That the two circulations are sufficiently separate to warrant the results being ascribed to the vaso-motor centre is shown by the fact that these reactions disappear after denervation or loss of reaction of the vessels of the part. The method for many investigations is an excellent substitute for the plethysmograph.

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The rate of diffusion into erythrocytes. By MONTAGUE MAIZELS.

In re-investigating the permeability of erythrocytes to phosphates [Maizels and Hampson, 1927] it was found necessary to determine the rate at which the anion diffuses. As the rate of phosphate diffusion varies with temperature, the temperature coefficient of phosphate diffusion was first studied. Heparinized blood was centrifuged and the cells mixed with one hundred times their volume of potassium phosphate solution of constant *p*H at various temperatures. After 5 minutes the suspensions were centrifuged in a series of tubes drawn out into sealed graduated capillaries. The tubes were then washed without disturbing the red cells in the capillaries. The volume of red cells was read and their phosphorus content estimated. At *p*H 8·4 and *p*H 5·4 the temperature coefficient between 10° and 20° C. was 1·7; between 20° and 30° C., 2·4; and between 30° and 40° C., 1·15.

Rate of Diffusion. If cells are suspended in mixtures of H_3PO_4, K_2HPO_4 and KH_2PO_4, $\Delta = 0{\cdot}42°$ C., the maximum phosphorus content after 1 hour at 24° C. is at *p*H 5·3. But if the phosphorus content is measured after only 5 minutes' exposure it will be found that this is maximal at *p*H 5·7. This indicates that phosphate permeates most easily at *p*H 5·7 (Table I).

If KCl ($\Delta = 0{\cdot}42°$ C.) is added to the phosphate it is found that the permeation of phosphate is greatly depressed; but the entry is most rapid at *p*H 6.

TABLE I.

	Cells in pure phosphate solutions			Cells in mixtures of potassium phosphate and chloride ($\Delta = 0{\cdot}42°$ C.)	
		Cell P			
*p*H	External P mg./100 cc.	5 min.	60 min.	External P	Cell P at 60 min.
3·6	402	78	275	—	—
5·1	396	160	340	198	32
5·3	390	180	352	195	35
6·0	367	192	325	183	68
6·2	358	155	290	179	60
6·7	337	105	230	168	45

When 0·05 c.c. of cells is suspended in a mixture of equal parts of KCl, and KH_2PO_4, the resulting *p*H is about 5·1, and the cell chloride is 112 millimoles, compared with 62 mm. in the external fluid. It appears that excess of chloride enters the red cell to balance the osmotic pressure of the external chloride and phosphate, and to satisfy the anion requirements of the red cell at *p*H 5·1. If cells are placed in a mixture of KCl (1 part) and KH_2PO_4 (5 parts), the external chloride is 21 mm. and the internal Cl 102 mm. This preponderance of cell chloride is brought about by an excess of any relatively indiffusible anion or non-electrolyte in the external solution.

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Impulses in the giant nerve fibres of earthworms. By J. C. ECCLES, R. GRANIT and J. Z. YOUNG.

In the ventral nerve cord of the earthworm there lie three very large non-medullated nerve fibres, and it has recently been shown that in the living worm the middle of these conducts antero-posteriorly and the two lateral fibres postero-anteriorly [Stough, 1926, 1930]. The fibres are not continuous throughout their length, but are divided into segments by membranes which apparently resemble the surface membranes of the fibres. The surfaces of contact are usually not flat, but curved in various ways so that the concave end of one segment fits over the convex end of its neighbour.

Impulses in the nerve cord can be recorded by a Matthews' oscillograph when the head or tail of the earthworm is stroked with a feather.

The conduction of such impulses has been studied in the isolated nerve cord stimulated by induction shocks. Two impulses are set up by a sufficiently strong shock applied to either the head or tail end of the nerve, the conduction rate of the faster impulse varying in different experiments from 17 to 25 metres a second and that of the slower from 7 to 12 metres a second (temp. 10° to 12° C.).

By altering the strength of the stimulus it can be shown that the impulses have an all or nothing relationship to this stimulus, even the finest adjustment of theshold failing to show that either impulse is made up of two separable impulses. Stough has described transverse connections between the lateral fibres, so it would appear that the presence of only two impulses is due to these two fibres forming a single conducting unit. By stimulating the nerve trunk first at one end and then at the other end a few sigma later and leading from the centre of the nerve to the oscillograph, it has been shown that the fast impulse in one direction travels in the same fibre as the fast impulse in the other direction, and similarly with the slow impulse. Thus, in spite of the transverse membrane, the impulses can pass in either direction. The unidirectional conduction found by Stough is not, as he suggested, due to the transverse membranes; it must be due to the connections of the nerve fibres.

At the head end the threshold stimulus for setting up the fast impulse is much lower than that for the slow impulse, while at the tail end the difference is always less. Also the action potential of the fast impulse at the head end is much greater than that of the slow impulse, but at the tail end the potential of the latter may even exceed that of the former. Since at this end the diameters of the lateral fibres are relatively to the median fibre, greater than anteriorly it seems certain that the slow impulse travels in the lateral fibres and the fast impulse in the median fibre. Experiments on the nerve impulse indicate that the rate of conduction is approximately proportional to the diameter of the fibre, and the action potential to the cross-sectional area. In our experiments the relative velocities of conduction of the fast and slow impulses are in accordance with the relative diameters of these fibres, but the action potential of the slow impulse is relatively about twice the size that would be expected from a single lateral fibre. This doubtless is due to the summed potential resulting from the two lateral fibres conducting as one.

The refractory period and recovery curve have been determined in three experiments, and are approximately the same for the median and lateral fibres. An impulse is conducted more slowly in the relatively refractory period and it has a lower potential. In fact the impulses in the

earthworm nerve have been very similar to the A group of impulses in frog nerve. In such respects, therefore, the transverse membranes have no influence on the conduction of impulses, although the separation of the segments by means of these transverse membranes appears to be as complete as that existing at vertebrate synaptic junctions. However, investigation into the polarization of these nerves by galvanic currents, though still in its preliminary stages, seems to bring out certain differences from vertebrate nerve which may be ascribable to the polarization of these membranes.

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The action of veratrine on frog's nerve. By H. Fromherz and A. V. Hill.

In muscle the prolonged veratrine contraction is associated [Hartree and Hill, 1922] with a prolonged liberation of heat, the ratio (at any moment) of tension to rate of heat production being the same as in an ordinary tetanus.

Nerves soaked in 1/50,000 veratrine and subjected to a short tetanus show a somewhat prolonged heat production: they also give a slight after-potential. In order, however, to obtain the characteristic veratrine effect we have first poisoned our nerves, then asphyxiated them in pure N_2 or H_2, then allowed them to recover in O_2. Graham and Gasser [1931] found this treatment to yield a prolonged after-potential. We have observed veratrine after-potentials lasting for minutes following a single shock to a nerve fully recovered from asphyxia. Presumably the drug is unable to penetrate and produce its characteristic effect until the nerve surface is somehow disorganized by oxygen want.

In a nerve veratrinized in this way the heat produced in response to a single condenser discharge may be 100 to 1000 times as great as in a normal nerve. In a succession of shocks at regular intervals the heat per shock is less, depending on the interval, but still far more than normally. The "veratrine ratio" is greatest after prolonged rest and grows less as the period since previous activity diminishes. The second of two shocks, *e.g.* at 5 sec. interval, may give half as much heat as the first, at 10 sec. interval two-thirds, at 30 sec. interval three-quarters.

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The effect on the body temperature of animals of injections of an extract of parathyroid glands. By M. H. B. Robinson and J. H. Thompson. (*Research Laboratories of the University of London, King's College and the Royal College of Surgeons, London.*)

It has been shown that extirpation of the parathyroid glands in rats and rabbits results in a disturbance of the heat-regulating mechanism; the injection of parathyroid preparations somewhat compensates for this loss of regulatory function [Ogawa, 1925].

A series of readings has been taken in rabbits and rats which have been injected with an extract of parathyroid glands, free from the factor which raises the blood calcium [Collip]. The preparation of the extract is described by Robinson and Thompson. The temperature readings were taken under standard conditions with regard to room temperature (65° F. thermostat controlled), diet, and time relation to injection. The thermometer was inserted per rectum sufficiently far to cover a fixed mark on the bulb. Daily observations were made. The results are tabulated as follows:

Range of temperature: average of 12 rabbits.

Rabbits.

	Weeks	1st	3rd	5th
Experimental	Average maximum	100	100·1	100·2
	Average minimum	99	99·2	99·3
Controls	Average maximum	101·5	101·2	102
	Average minimum	101	100·4	100·8

The average range of temperature during the experimental period was:

Experimental rabbits 99·1 to 100·1
Control rabbits 100·7 to 101·5

The experimental rabbits maintained their body temperature at a lower level than that of the control rabbits throughout the experiment.

Range of temperature.

Rats.

	Weeks	1st	2nd	3rd	4th	5th
Experimental	Average maximum	97·3	97·5	98	98·6	98·6
	Average minimum	97·2	97·1	97·2	98·0	98·0
Controls	Average maximum	98·2	98·9	98·6	98·9	98·7
	Average minimum	98·0	98·0	98·1	98·3	98·4

The average range of temperature during the period in which injections were made was:

Experimental rats 97·2 to 97·6
Control rats 98·0 to 98·6

Injections were discontinued after the end of the third week, and the

temperature of the experimental animals began to rise three days after cessation of injection. From that time up till the last readings the temperature of the experimental rats has closely approximated that of the control animals.

A preliminary experiment indicates that injection of extract of anterior lobe of pituitary gland can raise the temperature of animals in which it has been lowered by injection of a parathyroid extract as described.

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The effect of veratrine on the electrical response of crab's nerve.

By S. L. Cowan. (*Marine Biological Laboratory, Plymouth.*)

With a Cambridge Instrument Company's A and M galvanometer (sensitivity 1 mm. $= 4.10^{-10}$ amp. at 3 metres, and period 3·8 sec.) a spider crab's (*Maia squinado*) leg nerve gives an action current deflection of about 30 mm. in response to a single just maximal shock [A. V. Hill, 1932, and Beresina and Feng, 1932], provided by a condenser discharge.

Usually, after dissection, the nerve is allowed to rest for an hour in aerated sea water to avoid the onset of reversible inexcitability; in the present experiments it was transferred at the end of the first half hour to sea water to which had been added previously the required amount of veratrine hydrochloride solution, any carbon dioxide liberated being blown off by passing air through. With such dilute veratrine solutions as were used, 1 in 10 millions to 1 in 100 millions, the *p*H of the sea water need never be altered by more than 0·1 unit, and control experiments showed that nerves soaked in sea water, which had been acidified with hydrochloric acid and the carbon dioxide removed by blowing in air, gave responses which did not differ from those given by nerves soaked in untreated sea water. At the end of the second half hour the nerve was mounted, the peripheral end crushed and galvanometer connections were made *via* non-polarizable electrodes. After oxygen had been passed through the chamber for fifteen minutes to allow the nerve to recover from the crushing, readings were taken to get a base line and then the nerve was stimulated at the central end. The initial action current response of a nerve so treated with veratrine was very large, as much as

650 mm., and the area of the deflection time curve was increased more than a hundredfold. The "retention" of action current described by Levin [1927] and by Furusawa [1929] lasted for nearly half an hour.

The optimum concentration of veratrine, for autumn crabs, lies in the region of 1 in 25 millions to 1 in 50 millions. A definite fall in the response can be noticed with concentrations of 1 in 10 millions on the one side or 1 in 100 millions on the other; in the latter case the response of these non-medullated nerves is still about fifty times larger than normal. Asphyxiation after soaking in veratrine sea water and previous to stimulation in oxygen does not affect the response.

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Loss of heat at high altitudes. By Bryan H. C. Matthews.
(*Physiological Laboratory, Cambridge.*)

At sea level, in dry cold air, 15–25 p.c. of the total heat production of the human body is dissipated through the lungs and respiratory passages, in vaporizing water, setting CO_2 free from solution, and warming air. At high altitudes a much higher percentage of the total heat produced must inevitably be lost from the lungs, etc., for the heat loss per litre of respired air remains about the same while the potential heat gain in the form of oxygen becomes less and less. The rapid respiration necessary to obtain adequate oxygen may dissipate heat as fast as it can be gained by utilizing that oxygen.

If these effects are considered quantitatively some surprising results come to light. The heat loss per litre of air at $-10°$ C. respired (whether dry or saturated makes little difference) is approximately 27·5 cal. in water vapour, 2·5 cal. in CO_2 and warming air. *Thus total heat lost per litre of air respired is* 30 *cal.* From 1 c.c. of oxygen absorbed about 5 cal. can be produced. For each millimetre by which the tensions of oxygen in inspired and expired air differ, approximately 1·3 c.c. of oxygen are absorbed from each litre respired, and 6·5 cal. are available. *Total heat gained per litre respired and per mm. difference in oxygen tension in inspired and expired air*, 6·5 *cal.*

As the barometric pressure falls the oxygen tension in inspired air falls, that in alveolar air also falls, that of the expired air will always be a little above that of the alveolar sample owing to dead space, etc. With fall of barometric pressure the difference between the oxygen tensions in inspired and expired air becomes smaller and smaller.

It will be seen from the above figures that unless the inspired and expired air differ in oxygen tension by about 5 mm., even if clothing is so perfect that it prevents any heat loss from the skin, the body temperature cannot be maintained. By extrapolation from figures for alveolar air up to 23,000 ft. [Barcroft, 1925; Somervell, 1925] it appears that these conditions will be realized somewhere round about 30,000 ft. Human life above the point where this occurs will be impossible without respiratory aids or artificial heating, for the body temperature must fall.

I have constructed several forms of respirator to recover the heat and water vapour from air expired and return it to the inspired air; these are at present undergoing tests. It is hoped it may be possible to prevent 40–80 p.c. of the total heat loss at high altitudes, and to eliminate the throat irritation resulting from desiccation.

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Decreased urinary flow in rats on high fat diet. By Casimir Funk and P. P. Zefirow. (*Casa Biochemica, Rueil-Malmaison, France.*)

In connection with the work on the hormones appearing in urine [Casimir Funk and P. P. Zefirow, 1932] an interesting observation was made, which apparently so far has escaped the attention of investigators and clinicians. A series of rats were kept on a practically fat-free diet on one hand, and on a high-fat diet on the other. Without any noticeable difference in the water consumption in the two series of animals, and with the food consumption lowered only by 40 p.c. in average, the urine excretion was much lower in the high-fat series. In a few instances an addition of 125 g. of butter to the usual (but not controlled) diet in human beings caused a diminution of urine volume by about 20 p.c. No explanation can be offered at present, but it seems possible that a high-fat diet may influence the pituitary gland.

The animals used were rats, two males and one female kept together

in a metabolism cage. Altogether 48 young growing animals were used. Water and food were given *ad libitum*. The diets used consisted of 24 g. caseinogen, 62 g. starch, 10 g. dried yeast, 4 g. salt mixture, and 2 c.c. of cod-liver oil. In the high-fat diet 48 g. of starch were replaced by butter. In the last recorded experiment the fat content was increased to 73 p.c. and lard used instead of butter, without change in the results obtained. The first two experiments were performed during the heat of the summer, the last two in autumn and winter. In summer the urine volume was lower, due to increased loss through the skin. The results are summarized in the table below:

Exp.	Duration of experiment, days	Fat-poor diet: daily variations c.c.	Average c.c.	High-fat diet: daily variations c.c.	Average c.c.
I	8	22–30	26·2	1–6	3·3
II	14	20–53	32	1–11	3·7
III	10	14–34	26·1	11–24	14·2
IV	41	12–44	28·7	1·5–11	6·0

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Action of ether on the sympathetic system. By B. B. Bhatia and J. H. Burn.

Elliott [1912] showed that ether, chloroform and urethane caused a discharge of adrenaline from the suprarenals, which occurred so long as the splanchnic nerves were intact. We have investigated the action of ether on other parts of the sympathetic system, making our observations on cats from which the suprarenals were removed and using the decerebrate preparation with vagi cut, and also the spinal preparation.

When ether vapour is mixed with the air used for artificial respiration, there is a powerful contraction of the spleen, inhibition of intestinal movements and tone, inhibition of uterine movements and rise of heart rate. These effects are reversed as soon as the ether is discontinued. In animals in which the spinal cord is destroyed, these effects are not seen, except that a gradual paralysis of intestinal movements occurs which persists long after removal of the ether. The contraction of the spleen in the decerebrate cat is abolished when the sympathetic ganglia are paralysed by nicotine.

As shown by heart-lung experiments, ether weakens the heart more than is commonly supposed, but this is not obvious in the intact animal

because of a simultaneous vaso-constriction, presumably due to sympathetic impulses. Thus an amount of ether which has no effect on the blood-pressure of a decerebrate cat causes a profound fall of blood-pressure after paralysis of the sympathetic ganglia by nicotine. Since ether has no appreciable action on the blood vessels themselves, as shown by perfusion experiments, this fall represents the uncomplicated effect on the heart. It is concluded that ether causes a discharge of impulses along all sympathetic nerves.

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Electric symmetry of the thorax. By W. A. Osborne (*Melbourne*).

When the two hands are led off to the string galvanometer, as in the conventional lead I, and when the string is adjusted to give the sensitivity required for standard electrocardiograms, it will be found that normally a deep breath produces either a very small deflection or none at all. As is well known a slight movement of either arm or hand develops a current of several millivolts. Lack of symmetrical expansion of the thorax in deep breathing can be readily detected in this way, and it is rather curious that, though the diaphragm is not bilaterally symmetrical in the normal human being, no potential difference of any magnitude is produced. A remarkable fact is that all the left-handed students I have examined, both male and female, show on taking a deep breath a string deflection of several millivolts amplitude pointing to some disturbance of the normal balance of effort in the right and left halves of the thorax.

PROCEEDINGS
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The extraction of histamine from tissues by electrodialysis.

By R. G. MacGregor and W. V. Thorpe. (*Preliminary communication.*)

A simple method for the estimation of very small amounts of histamine in tissues would be of great value in studying the rôle of histamine in the body. Existing methods involve a laborious chemical extraction of the histamine from the tissue followed by a physiological assay of the extract. (Colorimetric estimation of histamine in tissue extracts by the Pauly reaction cannot be accepted as reliable.) The most convenient and reliable physiological method for the assay of histamine is matching against a standard solution of pure histamine on the blood-pressure of an anæsthetized cat.

Whilst our main aim, the substitution of a reliable chemical method for the physiological one, has not been achieved, we have effected a very considerable saving of time in the preliminary chemical manipulation. The alcohol method [Best, Dale, Dudley and Thorpe, 1927] requires attention during three days; the more drastic HCl method [Best and McHenry, 1930] requires one day. By our method of electrodialysis in a three-compartment cell histamine can be obtained from a tissue ready for injection within an hour and a half. The solution is clear and colourless whereas by the other methods it is coloured and often cloudy. No reagents apart from distilled water and a little acid for the final neutralization of the cathode liquid are necessary. The temperature can be kept below 35° C.

So far our experiments have only been concerned with blood and lung. In the former there is no histamine, but histamine added in varying amounts has been recovered practically quantitatively. In one experiment the histamine was easily isolated as picrate from the dialysate by drying with sodium carbonate and extracting with chloroform. In a number of experiments with lung (ox and dog) portions of the same mince were extracted by electrodialysis and by the alcohol method. In every case the dialysates and the control alcohol extracts had identical effects on the cat's blood-pressure. Although the histamine cannot be estimated by the Pauly reaction, the passage of histamine into the cathode chamber

has been followed by a modification of the reaction, in which *p*-nitraniline is used in place of sulphanilic acid. When all the histamine has been extracted the intensity of this Pauly reaction (measured in a Rosenheim-Schuster colorimeter) remains constant. The reliability of this indicator has been confirmed by assays on the cat's blood-pressure during some of the dialyses. The experiments, in addition to supporting the view that histamine in the tissues is either free or very loosely combined, confirm the reliability of the alcohol method used in previous experiments [Best, Dale, Dudley and Thorpe, 1927; Thorpe, 1928, 1930].

The apparatus is in essentials similar to that of Foster and Schmidt [1923], the cells being made of waxed wood to take 20 or 50 g. of tissue. The membranes are ordinary parchment dialysing paper and the cathodes of pure nickel sheet.

The experiments are being extended to other tissues and it is hoped to publish full experimental details on their completion.

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"Vaso-tonins" and the pump-oxygenator-kidney preparation.

By L. E. Bayliss and E. Ogden.

A. In order to discover the relative toxicity of heparinized and defibrinated blood, and the effect of time on the development of vaso-tonins, small quantities (about 10 c.c.) of the different bloods under test were added to a pump-lung-kidney preparation (containing 150–200 c.c. blood) and the rise in perfusion pressure recorded. The following conclusions were reached:

(1) Heparinized blood is not significantly different from defibrinated blood which has been properly filtered (through six thicknesses of butter muslin).

(2) The vaso-tonins develop immediately, *i.e.* within 2 min. of removing the blood from the animal.

(3) There is no significant increase in vaso-tonic activity with time up to 1½ to 2 hours, and it is immaterial whether the blood be kept warm or allowed to cool to room temperature.

(4) Up to 1½ to 2 hours after being shed, the rise of pressure produced passes off completely in 10 min. Blood kept for a longer time than this

produces a permanent rise in pressure which, as far as we can see, never completely passes off. These vaso-tonins, apparently, cannot be removed by a pump-lung-kidney preparation.

(5) Neither "temporary" nor "permanent" vaso-tonic activity is affected in any way by filtering the blood through fine cambric (50–70 μ pores).

(6) Defibrinated blood kept for 48 to 120 hours loses the "permanent" vaso-tonins, but retains the "temporary."

(7) Defibrinated blood which has been circulated through a pump-lung-kidney preparation regains its "temporary" vaso-tonins on being allowed to stand for an hour. It never develops "permanent" vaso-tonins.

(8) In the presence of a sufficient quantity of ergotoxin to inhibit the action of adrenaline (10 γ per 100 c.c. blood), almost any quantity of even the most toxic preparations can be added without resulting in any vaso-constriction (confirming the observations of Heymans, Bouckaert and Moraes [1932]).

B. If a kidney is to be perfused satisfactorily without simultaneous perfusion of lungs or other organs, the blood must be detoxicated by the kidney itself. We have accordingly constructed a perfusing circuit which, including the oxygenator, requires only 70 c.c. blood for an average-sized dog's kidney. The oxygenator consists of a piece of rubber tube about 1 metre long and 5 mm. inside diameter, connected between the outlet from the kidney and the blood reservoir. The reservoir is closed and connected to a vacuum pump, the vacuum being so adjusted that alternate drops of blood and bubbles of air (or oxygen) pass down the rubber tube. The uptake of oxygen is remarkably rapid, and the froth formed consists of large bubbles, and so breaks easily.

Kidneys perfused with this apparatus, however, never reach more than a half or three-quarters of the blood flow of these perfused in a pump-lung-kidney preparation, so that it seems probable that detoxication is never quite complete. The inclusion, from the beginning, of a fine cambric filter in the circuit did not result in any larger blood flow (in contradistinction to the observations of Daly and Thorpe [1932]). The urine flow, however, is usually quite good, and quite high concentration ratios may be obtained (exp. 5, on heparinized blood gave a maximum urine flow of 0·76 c.c. per min., and a creatinine concentration ratio of 30, the urine containing 0·09 *M* chlorides).

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A seven horse-power Austin engine adapted as a blood pump.

By I. de Burgh Daly.

The perfusion of the whole or portions of one or more large animals (dogs) in cross-circulation experiments necessitates larger perfusion pumps than those at present in general use. Two Dale and Schuster pumps in parallel are able to put out sufficient blood for perfusion of the whole systemic circulation of a small dog, but they are working to the limit of their capacity when it is desired to raise the systemic arterial pressure to a value higher than normal.

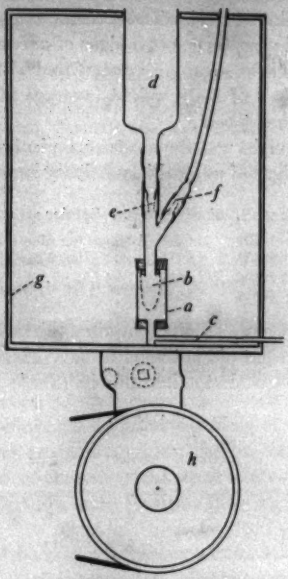
The apparatus described consists of four separate pumps, each of which is capable of dealing with considerable blood flows, and it has been found to be satisfactory for cross circulating large animals. A table is given below of the outputs at different pressures and frequencies.

Rate per min.	B.P. mm. Hg.	Maximum output of each pump, litres per min.
100	50	4·0
100	175	3·1
100	220	1·8
150	120	5·1
150	220	2·7

It will be seen that the output diminishes as the pressure rises, due to compression of the air in the transmission system, but except under certain circumstances this is not a disadvantage since the output of each pump can be adjusted whilst running. The figure shows one of the pump units mounted above the engine.

The inlet and exhaust port holes of the engine are closed and the four cylinders used as compressors by driving with an electric motor. The cylinders are in communication with glass chambers (*a*), containing heavy latex finger stalls (*b*) supplied by Macinlop, Manchester. The finger stalls are alternately compressed and decompressed by the rise and fall of the pistons, air transmission being used. The degree of compression and consequently the output of the pump are controlled by adjusting the opening of a side tube (*c*) communicating with the cylinders. The glass reservoir (*d*), inlet and outlet valves (*e*, *f*) are so arranged that no air is trapped in the pump circulation when blood is poured into the reservoir. The blood does not come into contact with any metal parts. The unit (*b*, *d*, *e*, *f*) is fixed by rubber-covered clamps (not shown in the figure) designed for quick removal for the purpose of cleaning. A glass-walled thermostat (*g*) kept at 37–39° C. surrounds the glass part of the pumps and is of sufficient size to prevent excessive local heating by the electric

lamps. The specification of the electric motor supplied by the Hockley Chemical Co. Ltd., is as follows: volts 220; amps. 2·5; H.P. 0·5; R.P.M. 500. A series resistance controls the frequency of the motor. A 2½ in. diameter flat pulley wheel drives on to the 9⅝ in. diameter engine fly-wheel (*h*) by means of a 1 in. wide flat belt. The glass connection tubes are ¼ to ⅜ in. internal diameter and the valves ⅝ in. flat rubber tubing.



Survival after adrenalectomy in rabbits. By CHARLES REID.

In a series of thirty-two rabbits, mainly of the albino Himalayan variety, adrenalectomized through the lumbar extraperitoneal route by aseptic operations at intervals of not less than 16 days, four died within 2 or 3 days, nine died between the 9th and 17th days with signs of adrenal insufficiency, and nineteen survived for long periods (over 100 days) still in apparently good health. This indicated that, in a series of successful double adrenalectomies, 60 to 70 p.c. of rabbits of the above species

may be expected to survive for an indefinite period. In this connection Kojima [1929] found accessory cortical tissue in 70 p.c. of normal rabbits. The above result is much better than that reported by Dominguez and Rogoff [1924] in which only three rabbits out of nineteen survived more than 30 days.

The adrenalectomized rabbits which died between the 9th and 17th days seemed normal on inspection until 2 or 3 days before death when loss of appetite, weakness and lassitude became evident. Before the onset of extreme terminal symptoms, other signs of adrenal insufficiency were (1) reduced fasting blood sugar p.c. as compared with normal; (2) hæmoconcentration; (3) loss of body weight (average 20–25 p.c. at death); (4) slow and feeble heart beat.

A fall in body weight was the most convenient and reliable guide to the probability of signs of adrenal insufficiency supervening.

TABLE I. Blood sugar p.c. Specific gravity.

Before adrenalectomy	0·102	1·048	Average for nine rabbits dying between 9th and 17th days after second operation.
After "	0·078	1·061	
Before "	0·106	1·050	Average for nineteen long-survival rabbits.
After "	0·098	1·052	

The series of thirty-two rabbits comprised thirteen males and nineteen females. Sex and weight did not seem to be a determining factor in the survival of double adrenalectomized rabbits (Table II).

TABLE II.

	No. of rabbits	Male	Female	Average wt. in kg.	
	32	13	19	1·45	1·50
Early deaths	4	2	2	1·42	1·49
Deaths from acute adrenal insufficiency	9	3	6	1·46	1·49
Survivals	19	8	11	1·45	1·51

In view of the frequent occurrence of œstrus in the rabbit and the striking prolongation of life after adrenalectomies reported in dogs in heat by Rogoff and Stewart [1928] in a small series of cases, this was not the result expected. It would be difficult in rabbits to attempt to establish the apparent relationship suggested by some writers between ovaries and adrenals in face of the already high percentage of survivals obtained in successful total adrenalectomies in both sexes.

Normal pregnancy and parturition with living young occurred in two adrenalectomized rabbits which were tested from the above series.

In a few rabbits, adrenalectomized apparently when immature, growth and increase of weight were observed. One male, for example,

increased in weight from 1·22 to 1·52 kg. in the course of 100 days after the second operation.

Signs, probably of chronic adrenal insufficiency, were seen after several weeks in four out of thirty rabbits in which one adrenal was removed and the other dealt with by denervation or medulliadrenalectomy for other investigations summarized previously [Reid, 1932]. They were some loss of weight and of hair (four), thickening and desquamation of skin (face and eyelids particularly) (three), flaccid paralysis of hind limbs (two).

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The future of the cathode-ray tube in physiology.

By H. Hartridge.

The cathode-ray tube could be employed in several present-day problems if records could be obtained at somewhat higher frequencies than those obtainable at present. Among these problems may be mentioned the calibration of fast chronaxie meters, the solution of the Wever-Bray problem, the accurate recording of speech sounds, noises, etc., and the accurate recording of impulses in composite nerve trunks. Possible directions of improvement are: brighter cathode-ray tubes, lenses of larger aperture, more sensitive films and greater rates of film travel.

With regard to the first, a brighter spot can be obtained on the end of the tube by using a higher gun voltage, but this causes in time disintegration of the fluorescent material. Two practical points may be made: (1) only to apply full voltage during the actual exposure, (2) to cause the cathode ray to travel successively over different parts of the fluorescent surface. This may be done by causing the cathode ray, which is oscillating horizontally, to move slowly vertically down the fluorescent surface. It may be practicable in the future to cause a film coated with the fluorescent material to move past the cathode ray inside the tube.

The lens system. I have pointed out elsewhere that it is advantageous for this to be of large aperture, and for it to project a reduced image of the end of the cathode tube into the camera. Cinema lenses, as at present

made, have not such large apertures as microscope objectives and condensers. There is a definite need for a special lens to be developed for cathode-ray work. Probably the best lens at present available is an achromatic substage condenser of 0·8 N.A., and about 1 in. focal length. Auxiliary lenses will be needed to flatten the field, and to reduce spherical aberration to a minimum. As made at present, the cathode-ray tube presents a convex surface towards the lens. It would be much better for this surface to be concave. This might be achieved by coating a piece of glass of suitable contour with the fluorescent material, and for this to be mounted inside the cathode-ray tube.

The camera. Super-speed cinema film is available in 16 mm. size. It would be difficult to pass this through the gate of an ordinary cinema camera at speeds of between 10 and 100 metres per sec. An alternative method is to wrap the film round a rapidly rotating drum. A drum of 16 cm. driven at a sped of 20 to 200 revolutions per sec. would provide the required speed. The record obtained would be $\frac{1}{2}$ metre long, and would be photographed in 1/20 sec. If longer records are required they could be obtained by winding the film spirally round the drum, and arranging for the lenses to move along sideways, so that the image should fall on the centre of the spiral as the drum rotates. Ten spirals would provide a 5 metre record of $\frac{1}{4}$ sec. duration. A possible future development is to cause the cathode ray to fall directly on suitable sensitized photographic material, the whole camera being exhausted prior to the exposure.

High-speed chronaxiemetry. Since spring-driven chronaxiemeters may have arm speeds of about 10 metres per sec., film speeds of 10 to 100 metres per sec. are required for the calibration of such instruments. It may be possible in the future to make the cathode-ray tube itself act as a chronaxiemeter. This may be explained as follows: the cathode "ray" consists of negatively charged electrons, and when these fall on a metal surface, a current flows to this surface from earthed parts of the electrical circuit. Consequently, if one electrode be connected to this plate, and the other to earth, a current flows during the time that the cathode ray falls on the plate. The strength of the current depends on the number of the electrons received in unit time (the "intensity" of the beam), the duration of the current depends on the size of the plate and rapidity of the swing of the cathode-ray. It is known that very rapid movements are possible and therefore very high speeds should be available.

General laboratory purposes. As at present made cathode-ray tubes are large in size, their filaments require a heavy current, and high voltages on

"the gun" are required to successfully work them. Future improvements are likely to reduce these and so make them available for the general measurement of voltages and currents. Since they have no moving parts they would have great advantage over existing types of instruments.

The avoidance of ketosis in the cleidoic egg.

By Joseph Needham.

The excretion of aceto-acetic acid and β-hydroxy-butyric acid is associated, as has long been known, with starvation, severe diabetes, and the restriction of carbohydrate intake. Of these three conditions, the third is eminently observable in the diet of the chick embryo, especially during the last half of its incubation period. On the 17th day of development, for example, the embryo absorbs 16 mg. of carbohydrate, 396 mg. of protein, and 528 mg. of fat (data in Needham [1931 *a*]). Such a diet should be highly ketogenic, and in fact the "ketogenic balance," calculated according to the method of Hubbard and Wright [1922], is in the neighbourhood of 30 during the last week of incubation. In man the threshold of ketonuria seems to occur at a ratio of 85.

In spite of this very unbalanced diet, however, the chick embryo develops no ketosis. A systematic examination of the allantoic liquid at all stages of development after the 10th day by Rothera's test never gave any indication of the presence of aceto-acetic acid, although the reaction is very sensitive. As ammoniacal nitroprusside does not give evidence of β-hydroxy-butyric acid, and as this acid is often quantitatively the most important of the keto-bodies, further experiments were made in which the van Slyke method [1917] (oxidation to acetone with bichromate and formation of the insoluble mercuric sulphate compound) was applied to allantoic liquid. Here also the results were negative, although small quantities of keto-acid added to the allantoic liquid could be quantitatively recovered.

It might be argued that ketosis depends not so much upon what is absorbed as upon what is combusted. This is not so easy to ascertain, but the embryo, which in the last week of development is 80 p.c. of the living matter in the egg, has certainly then an R.Q. of 0·7, the allantois an R.Q. of 0·8, and the yolk-sac an R.Q. of 0·65 or less. The predominance of fat combustion in this period can hardly be doubted. If, then, it be admitted that the chick avoids ketosis under conditions amply sufficient to provoke it in the mammal, the mechanism of this avoidance becomes a question of great interest. It may be mentioned that a search of the literature has

failed to discover any case of ketosis in an adult bird, even in diabetes. It is possible that immunity to dietary ketosis may have to be added to the list of special adaptations characteristic of "closed box," or cleidoic, development—suppression of protein catabolism, intensification of fat catabolism, uricotelic nitrogen-excretion, and relative shortening of incubation time [Needham, 1931 *b*]. Work on the problem is in progress.

REFERENCES.

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